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DANIEL TREMBLY MACDOUGAL

PIONEER PLANT PHYSIOLOGIST*

(WITH ONE PLATE AND TWO FIGURES)

It was about fifty years ago and at DePauw University, where he was at that time a junior student assistant, that the young DAN MACDOUGAL undertook, with only a window-sill for a laboratory, to follow some of Professor J. C. ARTHUR's typewritten directions for experiments in plant physiology. He was apparently successful, for the next year found him at Purdue University, working as instructor in Professor ARTHUR's laboratory. With Professor ARTHUR's encouragement—and in spite of the admonitions of one or two others, who thought there was "no future for a young man in plant physiology"—he continued to show more interest in physiology than in other aspects of botany. After three years at Purdue, he moved on to take charge of his chosen subject at the University of Minnesota, where he remained till 1899.

With leave of absence for 1895–96, MACDOUGAL studied at Leipzig with WILHELM PFEFFER and visited the laboratories of JULIUS SACHS, HERMANN VÖCHTING and other leaders in Germany, Holland and England. Research begun with PFEFFER led to an important contribution on "The Curvature of Roots," on the basis of which Purdue University conferred on MACDOUGAL one of the three Ph.D. degrees ever awarded by that institution to persons not in residence.

Three years after MACDOUGAL's return from abroad he left Minneapolis to become director of the laboratories at the New York Botanical Garden. In that capacity—later as assistant director and at times as acting director of the New York Garden—he contributed much in an administrative way to the fine organization of that institution, but there was no let-up in his al-

* The American Society of Plant Physiologists dedicates this issue of PLANT PHYSIOLOGY to DANIEL TREMBLY MACDOUGAL, in celebration of the occurrence of his seventy-fifth birthday, on March 16, 1939.—*Committee on the* MACDOUGAL *Celebration:* FRANCIS E. LLOYD, HOMER L. SHANTZ, CHARLES A. SHULL, BURTON E. LIVINGSTON.

ways vigorous attacks upon fundamental scientific problems. Also he gave generously in helpful suggestion and encouragement to those advanced students who were so fortunate as to spend portions of their formative periods in his laboratories.

It seems that MACDOUGAL had begun to accumulate his great fund of observational knowledge about desert plants and their environments as early as 1891, when he devoted the summer to botanical collecting in Arizona, under the auspices of the U. S. Department of Agriculture. In his years at Minneapolis and at New York he made a number of field excursions to study the plants of the American Southwest. Thus he was ready to give enthusiastic support to H. V. COVILLE'S suggestion that the newly founded Carnegie Institution might well undertake special botanical investigation in that region. He served on the board whose recommendations and plans led to the establishment of the Desert Laboratory, near Tucson, in 1903, and it was natural for him to become the first director of the new laboratory as well as of the Department of Botanical Research of the Carnegie Institution of Washington, positions which he held from 1906 to the time of his official retirement in 1928.

At the Desert Laboratory and later also at the Coastal Laboratory—at Carmel, California—MACDOUGAL carried out many long-time studies and experiments with plants in their natural setting, as well as much laboratory experimentation, all in the general field of plant physiology, ecology and plant geography. With a staff of able and enthusiastic colleagues, he developed his department to a high degree of versatility and effectiveness. Visiting investigators were always more than welcome and were furnished with whatever materials and facilities were requisite for what they wished to do. He conducted many excursions into regions of special ecological interest—among the mountains, plains and valleys of Arizona, southern California and northwestern Mexico. In company with GODFREY SYKES, he spent a period in the Libyan desert. Observational knowledge and experience gained in the field were continually brought to bear on physiological experimentation in laboratory, shop and experiment garden. He has turned again and again to problems of plant water relations, of growth and enlargement, of imbibition and swelling. He invented, improved and brought into use several auxographic instruments of precision, the best known of which is the MACDOUGAL dendrograph, an apparatus for the automatic recording of volume changes in tree trunks. By its means he has contributed very greatly to our knowledge of growth in trees. His recent book, on "Tree Growth," summarizes eighteen years of persistent study.

While engaged in all these varied scientific and administrative activities, MACDOUGAL has found much time and energy to devote to the organization of American science workers. For many years he has been active in the

several special societies that deal with plant science, also in the American Association for the Advancement of Science. He was active in the development of the Pacific Division and of the Southwestern Division of the American Association. At the Indianapolis meeting of the American Society of Plant Physiologists (December, 1936) he was elected to a Charles Reid Barnes life membership in that society.

It is quite impossible to sketch adequately, even in faintest outlines, the character and accomplishments of DANIEL TREMBLY MACDOUGAL, whose interests have been so wide, and yet so discriminating, and whose contributions have been so valuable in so many ways. All who know him for his work and all who love him for his genial personality will surely join me in urging him to keep on for many more years, continuing to enlarge and clarify the fields of knowledge which he has already so devotedly and successfully illumined.—GEORGE T. MOORE.

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Although MACDOUGAL was necessarily kept very busy with a large amount of administrative work while at the New York Botanical Garden, he was always ready to discuss botanical problems and research projects with any one of his colleagues or students who wished to undertake or to continue promising investigation. His friendly personality, his thorough general knowledge of botany and his great enthusiasm for research made his New York laboratory, on the upper floor of the Museum Building, in Bronx Park, a very pleasant place in which to work. He set a fine example to all about him, for he was always engaged in scientific projects of his own, from which he generally secured valuable scientific results for subsequent publication. An early riser, he was often seen about the garden, greenhouses and experiment grounds when gardeners and workmen were just beginning their day; also, it was in the early morning that he accomplished much of his writing. Inveterate experimentalist and laboratory worker that he was, he also keenly loved the out-of-doors and liked to devote holidays to short field excursions with a few companions, with whom many things might be discussed by the way. One who held a research scholarship at the New York Botanical Garden at that time loves to recount how he accompanied MACDOUGAL on a memorable tent-camping excursion of several cold and snowy days at the end of November in 1903, to the barrens and marshes inland from Barnegat, on the New Jersey shore.

In his New York period MACDOUGAL published his monograph on the formative influence of light on plants, which gives accounts of many ingenious experiments and a wealth of valuable new observations. When he undertook the important task of editing for publication the California lectures of HUGO DE VRIES on the mutation theory (HUGO DE VRIES. Species

and Varieties, etc. Ed. by DANIEL T. MACDOUGAL. Open Court Publ. Co., Chicago, 1905.), he was already very familiar with many of DE VRIES's new ideas and experimental methods. He had established an experiment garden at the Bronx as early as 1902 and had there been studying mutation in his own cultures of the great evening primrose. That small experiment garden —only about half an acre in extent—was apparently the first in this country to be devoted to studies of this kind. DE VRIES pointed out that this American student had furnished additional proof of the validity of the mutation theory. The publication of these lectures first brought the new theory clearly before English-speaking botanists. In a lecture given before the Barnard Botanical Club, of Columbia University, on December 18, 1905, just before he left the New York Garden for the Desert Laboratory, MACDOUGAL made what appears to have been the earliest announcement of a successful attempt to alter inheritance or induce mutation by means of experimental treatments of plant ovaries. Referring to his injection experiments with Raimannia ovaries, he pointed out that "agencies external to the cell may induce mutations, and consequently exert a profound influence on heredity."

Realizing that one of the prime requisites for productive outdoor studies on plant growth and reproduction is definite knowledge about the influential conditions of plant environments, MACDOUGAL installed the requisite instruments at his experiment garden and accumulated for several years detailed records of precipitation, air humidity, air temperature and soil temperature. He was among the first to try to study these environmental conditions quantitatively, especially soil temperature, with reference to plant performance. His keen interest in this climatological aspect of physiological ecology has been maintained throughout his subsequent years of productive investigation. He began to devise and improve climatological and auxanometric recorders of several kinds while at New York, showing a remarkable degree of native ingenuity in that field of research.

From New York MACDOUGAL made a number of brief excursions into the arid Southwest, bringing back plant material and many living plants to be grown in the New York Garden. It was clear to every one at that time that the acute and energetic mind of the director of the laboratories was greatly attracted to problems concerning the ecology and physiology of cacti and other plants of arid climates. His interest in desert plants increased and bore fruit, until, as the leading American authority on desert ecology, he was almost suddenly transplanted from New York to Tucson, to become director of the Desert Laboratory and of the Department of Botanical Research of the Carnegie Institution of Washington.—C. STUART GAGER.

Alone and on foot, with a single burro to carry the packs and with camp equipment consisting of just "a Navajo blanket, a tin cup and a jar of beef extract," MACDOUGAL spent most of the summer of 1891 collecting plants in northern Arizona. The experiences of that summer must have stimulated a native pioneering instinct and traits of alertness, resourcefulness, originality and capacity for prompt decisions, which have distinguished the effective investigator and administrator whom we know. The Flagstaff country was revisited in 1898 and the regions of Tucson, Torres and Guaymas were visited in 1902. He returned to the mountains and valleys about

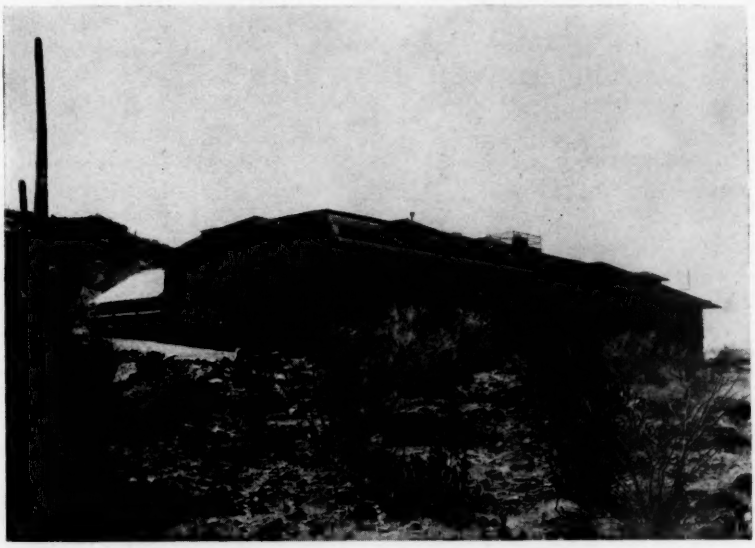

FIG. 1. Main Building of the Desert Laboratory, 1911.

Tucson in 1899, when he and F. V. COVILLE selected the location for the projected Desert Laboratory, on behalf of the recently founded Carnegie Institution of Washington. The first building of the new laboratory was erected in 1903 and MACDOUGAL visited it in the spring of 1904, extending his excursion at that time to the delta of the Colorado River. In the late winter of 1905 he and GODFREY SYKES began their long-continued studies of the delta region. Thereafter he devoted much study to the recently flooded Salton Basin, which he visited at least twice each year for a number of years.

Well equipped with general and special knowledge concerning the plant geography and ecology of the American Southwest, and with unbounded

enthusiasm for further studies in that region, MacDougal became Director of the Department of Botanical Research of the Carnegie Institution of Washington at the beginning of 1906, when he migrated from New York to Tucson and made the Desert Laboratory his official headquarters. The recently erected building, high on a shoulder of Tumamoc Hill, was about three miles away from what was then in many respects a frontier town, and the development of facilities for scientific work involved many difficulties.

Fig. 2. The Director of the Desert Laboratory, 1923.

Transportation was slow, one walked or rode horseback, and goods were brought from town by team. The primitive road and the water supply required immediate attention. A three-room addition and a small greenhouse were promptly added to the original building, and the extensive grounds were fenced. Climatological instruments were soon installed and a small working library was assembled. All such duties and difficulties were im-

mediately faced by the new director with vigor and efficiency, and several important research projects were at once started.

MACDOUGAL'S deep and friendly interest in the members of his staff was constantly sustained; he responded generously to all requests for equipment, which were naturally numerous and extremely varied. He liked to have about him fellow workers rather than assistants—staff members and visitors who were not dependent but who told him what they intended to do next. He was generous with helpful suggestion but he never gave commands and there was never a more loyal or more cooperative group than the one he contrived to have about him. He was remarkably successful in engaging the whole-hearted cooperation of many investigators in other laboratories.

The director was habitually first to appear in the morning and he accomplished most of his writing before the rest of us were up. One of his secretaries, remorseful at always finding upon his arrival in the morning that the director was already busily at work, came earlier and earlier for several days, but when he was at length able to reach the office before MACDOUGAL the latter seemed more annoyed than pleased. Routine office work was quickly dispatched but there was always time for a cordial correspondence with other science workers throughout the world. He readily grasped the views of others and his judgments concerning their ideas were always appreciative and friendly as well as keenly critical.

With equal talents for laboratory experiment and field observation and with equal interest in them, MACDOUGAL'S first aim seems to have been to assemble and evaluate many widely different kinds of knowledge so as to make possible a true appreciation of plants and their activities as they occur in nature. Now and again he would go away with one or two companions—by saddle and pack train, by team or by automobile—for a day or more of camp life in the nearby mountain ranges or on the great bajadas of the Tucson region. Such enjoyable excursions, pleasantly remembered by many visitors at the Desert Laboratory as well as by staff members, always resulted in new observations and interpretations, and they were recreational in the best sense. It was on field trips of this kind that one discovered one's host to be a grand master of outdoor cookery; he specially liked to broil a steak "the size of a vest-pattern" over a bed of coals.

MACDOUGAL is now residing near Carmel, California, where, freed from administrative responsibility, he is continuing his studies, especially on tree growth, with the same ingenuity and keen enthusiasm that characterized the years before his retirement. His productive activity has shown no let-up, his work shows no signs of being finished, he is the same MACDOUGAL whom we have known these many years.—FORREST SHREVE.

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CARBOHYDRATE AND RESPIRATORY METABOLISM IN THE ISOLATED STARVING LEAF OF WHEAT

G. KROTKOV

(WITH TWELVE FIGURES)

Introduction

From the point of view of its respiratory constitution the foliage leaf of the wheat plant is an example of an interesting and somewhat special type of plant organ. The essence of its constitution is the possession of a high potential enzymic activity relative to its low primary reserve of substrate materials, and consequently the wheat leaf passes through its ontogeny rapidly and has a brief duration of life. A leaf of this type contrasts strongly with such organs as potato tubers, or apples, which stand at the opposite extreme, having substrate reserves which are enormous in relation to their potential enzymic activity. As a result such organs maintain themselves for long periods of time and their physiological state changes very slowly.

From numerous observations by different workers on all kinds of plants and detached organs which respire at a rate proportional to their sugar content it has been concluded that sugars serve as the usual respiratory substrate. It must be noted, however, that the great majority of such observations are of short duration and are usually made on material which has just been removed from its natural surroundings. Consequently, such observations apply to respiration in the initial stages of starvation only.

ONSLOW, KIDD, and WEST (**11**) using storage apples as experimental material, followed changes in sugar content and respiration for about 150 days. Over this interval of time, or any part of it, they found that CO_2 output was roughly equivalent to the total sugar loss. It may be concluded that in apples sugars serve as the respiratory substrate for at least 150 days in storage. Similarly BARKER (**1**), working with potato tubers in storage, found sugars to serve as the only respiratory substrate. In addition, by exposing tubers to cold for various lengths of time, this investigator brought about variations in the sugar content of tubers between 0.2 per cent. and 7.0 per cent. and then studied the corresponding changes in their respiration. By plotting respiration rates of tubers against their sugar contents he found that the curve so obtained had the form of an "enzymatic rectangular hyperbola," the usual form of relation between the rate of an enzymic reaction *in vitro* and the concentration of its substrate.

It appears then that sugars serve as the respiratory substrate in various plant organs immediately after their separation from the plant, and in organs of certain type, for a long period afterwards. From BARKER'S re-

sults one would expect to find the basic relation between respiration rate and sugar to be hyperbolic, but often much obscured by interfering circumstances.

Much less is known of the effects of depletion of this primary substrate on plant metabolism. The fullest account of these effects is found in the work of DELEANO (**4**). As experimental material this worker used grape vine leaves. On detachment from the plants half of the leaves was used at once and the other half was kept in darkness for various lengths of time. The CO_2 emitted from these leaves was measured and at the end of the various periods of starvation, leaves were analyzed. It was found that for any interval of time within the first 5 or 6 days all of the CO_2 produced by the leaves was roughly equivalent to the sugars lost, on the assumption that these sugars were completely broken down into CO_2 and water. Within this first period there was no appreciable degradation of proteins. After 5 or 6 days proteins began to disappear from the leaves, and the CO_2 evolved was then in excess of that which could have been derived from the sugars simultaneously lost.

Unfortunately no continuous records of respiration are given in DELEANO's paper, so that it is impossible to say whether this degradation of proteins is correlated with any marked changes in the respiratory rate such as occur in other starving leaves and which are so typical of the respiration record in the first foliage leaf of wheat. After the experiments reported in this paper had been completed a paper was published by YEMM (**13**), who, working with starving barley leaves, reached the same conclusions as DELEANO as to the nature of the respiratory substrate on prolonged starvation. But the respiration records reported by YEMM are of a different type from those of wheat, and the large hump which is so characteristic of the respiration record of wheat is lacking or highly modified in barley. Clearly then, data on the relation between respiratory activity and the substrate materials on prolonged starvation in organs of the wheat-leaf type are lacking. The experiments reported in the following pages are intended as a contribution toward the rectification of this defect.

Materials and methods

The data treated in this paper are the results of four experiments in which changes in the rate of CO_2 emission and changes in the sugars were concurrently determined during the starvation of the isolated, first foliage leaf of *Triticum compactum* Host. variety Little Club. The four populations of seedlings were all grown from the same lot of seed. This was a pure line of Little Club obtained from two sources: the Rust Research Laboratory in Winnipeg, and the Bureau of Plant Industry in Washington. Seed from the two institutions was mixed in the proportion 4 to 9. All the

experiments conducted are numbered in chronological order and will be referred to by number throughout the paper. In each experiment plants were grown in soil until their first leaves reached the stage of "early maturity" at which point a large stock of leaves was detached and used for experimentation.

It has been found by DUFF and FORWARD (5) that, in the course of their metabolic drift with age, wheat leaves grown under standard, artificial conditions enter the stage of early maturity at a little under two weeks from the time of planting. The significant known characteristics of this stage are as follows:

1. The leaves have attained their maximum length.
2. The leaves are active in photosynthesis and the endosperm reserve of the seedling is exhausted.
3. The initial respiration rate when detached, has fallen sharply from high values of the order of 100 mg. of CO_2 per 100 gm. of leaf fresh weight per hour, which are characteristic of the early stages of growth, and has reached a more nearly stable value of about 35 mg., from which it very gently declines for the several weeks during which the state of maturity is maintained.
4. The leaves contain no starch.

The conditions under which the seedlings were grown before experimentation were different in all the experiments. In experiment 1, the plants were grown under the standard conditions described by DUFF and FORWARD (*loc. cit.*). On the other hand, the plants used for experiments 2, 3, and 4 were grown in the summer in the greenhouse where no attempt was made to regulate external conditions, and where both temperature and light varied considerably. It was not surprising to find that the seedlings grown for the different experiments differed from one another in a number of ways. The most obvious difference was that of their sugar content, which in experiment 1 was several times greater than in experiments 2, 3, and 4. Though there was more similarity among the three populations grown in the greenhouse than there was between any one of them and the plants grown under artificial conditions, nevertheless, plants from the greenhouse were not identical. The sugar content of the leaves in experiments 2 and 4 was nearly the same, but was considerably lower in experiment 3. It would be reasonable then to expect that in plants grown under such different conditions, the physiological state should turn out to be different if appropriate criteria of comparison could be developed.

On the day when the experiment was to begin, after a period of prolonged illumination, the entire population of seedlings was brought to a sufficient degree of homogeneity by removal of the extreme variants with respect to height. From these selected plants the first leaves were detached at the ligule and placed in a beaker with distilled water. The stock of leaves so isolated served as the actual experimental material and from it the fol-

lowing samples were taken: (1) A sample of about 50 or 75 leaves for the respiration determination; (2) a sample of 50 leaves for the first sugar analysis; (3) two samples of 50 leaves each in experiments 2, 3, and 4 for determinations of the changes in fresh weight during starvation. The remainder of the stock of leaves, after taking the above described samples, was set aside standing in water at the temperature of the respiration experiment, and used for subsequent periodic sampling for sugar analyses.

The sample for the respiration experiment was immediately weighed, transfererd to the respiration chamber, and the determination of the CO_2 begun. The sample for sugar analysis, after weighing, was cut at once into small pieces and dropped into boiling alcohol for the extraction of sugars. The initial weights were taken of both samples for the determinations of the leaf fresh weight, and then both samples were placed in two separate beakers with the rest of the stock of leaves, in the dark at the temperature of the respiration chamber, where they were kept till the end of the experiment, their fresh weights being taken daily.

The determinations of respiration rates were extended continuously over the whole period of starvation, which required about 5 days for completion. During the whole of this period the leaves were standing in a glass respiration chamber with a few ml. of distilled water at the bottom. The respiration chamber was kept in darkness in a constant-temperature water bath with temperature variations not exceeding 0.25° C. for the whole period of starvation. In experiment 1 the temperature of this bath was kept at 22.3° C. and in experiments 2, 3, and 4 at 20.0° C. By means of a clock mechanism the current of air, continuously drawn through the respiration chamber, was switched to a new Pettenkoffer tube every 3 hours in experiment 1 and every 4 hours in experiments 2, 3, and 4, so that in each experiment a continuous record of respiration was obtained.

In a wheat leaf, starch can be found only in the early juvenile stage and it disappears later, on entering into the stage of early maturity (**5**). It has been assumed then that in this last stage no degradation products of starch, such as maltose, can be present. This assumption is fully supported by NEWTON (**9**) who, working with wheat leaves in various stages of their development, found no complex sugar other than cane sugar. It should be remembered, however, that this worker used leaves which were not strictly comparable with the leaves used in the present work. The writer is not certain that the sugars found in earlier stages of development of the first leaf are necessarily the same as in the later stages. Work is being carried on now on the isolation and identification of the various sugars from wheat leaves and until this is completed no final statement can be made as to the nature of the sugars present. It should be remembered, however, that the leaves dealt with in the present experiments were all in the same stage of their physio-

logical development and consequently would not differ from one another as a result of variation in sugars with age. Moreover, for the present purpose it is not necessary to define exactly what sugars are present. It is sufficient to divide all of the alcohol-soluble carbohydrates of the wheat leaves into two fractions based on their reducing power before and after inversion. In the first fraction, under the name of "simple sugars," are put all those sugars which showed reducing power before acid inversion. In the second fraction, under the name "invert sugars," are included those carbohydrates which showed reducing power after inversion with acid. The amounts of the various carbohydrates present in both fractions have been calculated as glucose. The methods adopted for the extractions of sugars from the leaves and for the subsequent preparation of the alcoholic extracts for the sugar analyses were essentially those of DAVIS, DAISH and SAWYER (3). Into their technique two modifications were introduced. First, in all the experiments the time of extraction has been extended to about 30 hours. This modification has been adopted on the strength of results from several preliminary experiments which have indicated that to remove the bulk of sugars from the leaves it is necessary to carry out extraction for many hours. In one such experiment 13.7 gm. of wheat leaves 12 days old from planting were extracted with four changes of 43 per cent. ethyl alcohol which was made 0.005 N with respect to ammonia. The four alcoholic extracts so obtained were converted into aqueous extracts and finally analysed for their sugar content before and after inversion with HCl. The results of analyses are given in figure 1.

The second modification introduced was that all the inversions of reserve saccharides present in the extracts were carried out not by means of invertase or citric acid, but by means of HCl according to the method adopted by the Association of Official Agricultural Chemists (10).

The procedure, so modified, was then briefly as follows: a sample of leaves was quickly weighed, cut into small pieces, and dropped into 250 ml. of boiling 85 per cent. ethyl alcohol which was 0.01 N with respect to ammonia. After about 30 hr. of boiling in a water bath under reflux condenser, the contents of the extraction flask were transferred to an Erlenmeyer flask, a few ml. of toluene were added, the flask was stoppered tightly and stored in the dark until the preparation of the extract for analysis could be carried further. The extract was then filtered from the leaf residue and the alcohol was evaporated under diminished pressure at a temperature not exceeding 45° C. The residue was taken up in water and cleared with basic lead acetate. The excess of lead was removed as carbonate, the filtrate was made up to volume, adjusting its reaction with NaOH to a value slightly above pH 8. In this form the extract was employed for analysis, its reducing power being determined by the original

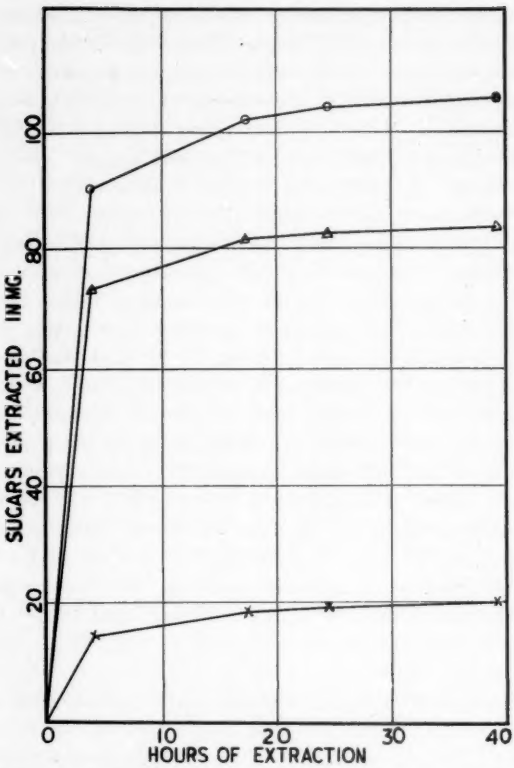

FIG. 1. Absolute amounts of sugars removed from leaves on prolonged extraction ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars.

HAGEDORN-JENSEN method (8) before and after inversion with HCl. The actually determined quantities were therefore the reducing sugars (before inversion) and the total sugars (after inversion). The invert sugars were estimated by difference.

Results

The respiration records of the 4 experiments are given in figures 2 to 5. Experiment 1 was carried out at 22.3° C. and the others at 20.0° C. The records have been plotted without correction for the temperature difference. Temperature corrections have been made wherever comparisons of rates at single temperatures are needed, the temperature-respiration relation having been worked out by DUFF (6) over the range from 10° to 30° C.

The records are all of the striking general form some modification of

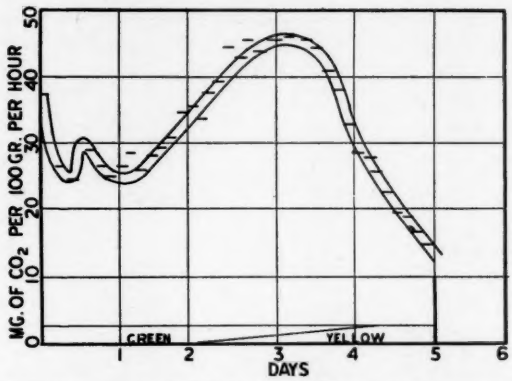

FIG. 2. Respiration record of detached wheat leaves during starvation. ═══, respiration record[1] (from exp. 1).

which is characteristic of the starvation respiration of the isolated leaves of many species. Respiration starts out with a high rate and declines from the beginning to considerably lower values. These give way without appreciable pause to rising values which reach a peak on the third day and from

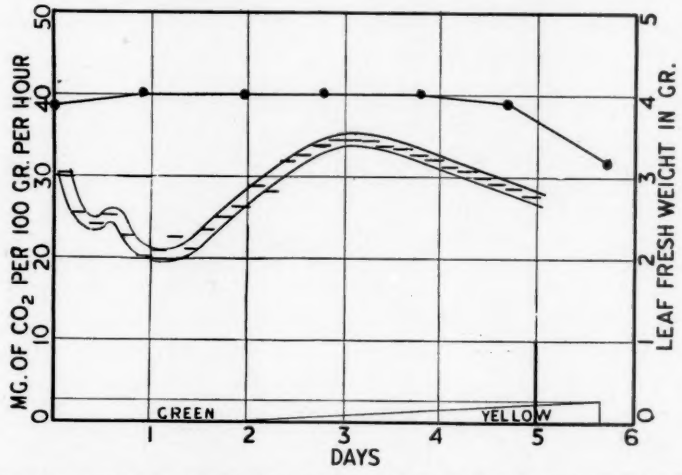

FIG. 3. Respiration record of detached wheat leaves during starvation. ═══, respiration record; ●—●—●, changes in the leaf fresh weight (from exp. 2).

[1] The author wishes to express his thanks to Dr. G. H. DUFF and Miss D. F. FORWARD for their kind permission to reproduce this respiration record, which was obtained by them in the course of their own work.

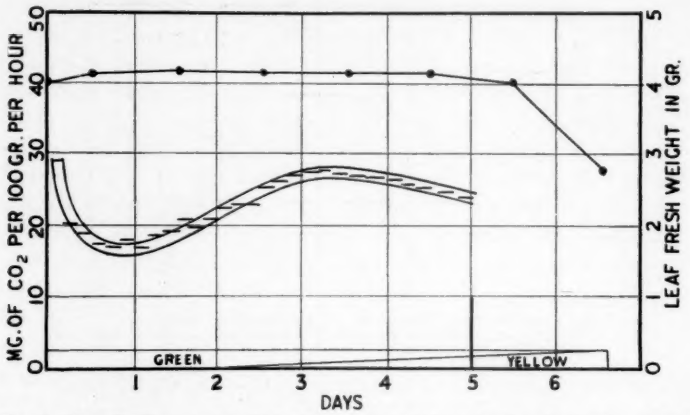

FIG. 4. Respiration record of detached wheat leaves during starvation. ═══, respiration record; ●—●—●, changes in the leaf fresh weight (from exp. 3).

that point onward again decline. These variations in the rates of CO_2 emission are correlated in time with the visible changes taking place in the leaves. The green color first pales slightly, is then replaced by yellow, which finally gives way to darkening with exudation of sap into the intercellular spaces. A chart representing the time sequence of these changes is given with each respiration record.

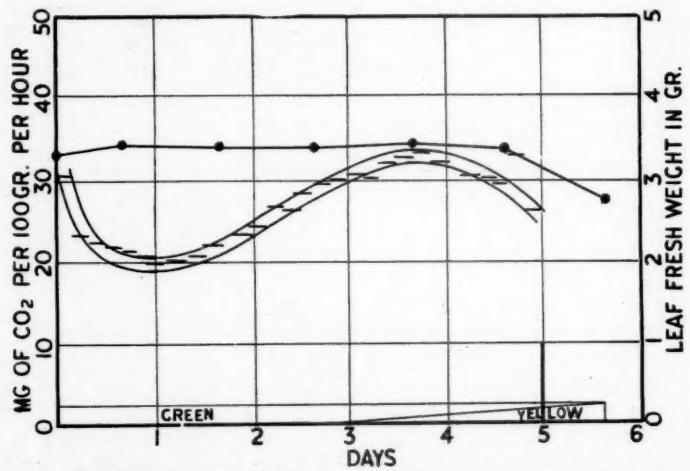

FIG. 5. Respiration record of detached wheat leaves during starvation. ═══, respiration record; ●—●—●, changes in the leaf fresh weight (from exp. 4).

The final condition is obviously one of autolytic disorganization. Until it sets in, the fresh weight of leaves does not alter appreciably. As long as leaf weight remains constant it is clear that the permeability properties of the leaf cells have not been so altered as to impair their osmotic action and thus the fundamentals of cell organization are retained. With the appearance of darkening in color and exudation of sap, the leaf weight falls sharply. These are all coincident evidences of the onset of the final autolytic disorganization. At the same time CO_2 emission becomes irregular in rate and thereafter can no longer be regarded as the product of an organized respiratory mechanism. The starvation respiration records are therefore considered to end at this point. Graphs showing fresh weight plotted against time are included with the respiration records in the three experiments in which fresh weight determinations were made. The slight initial rise which characterizes these graphs represents, no doubt, absorption of water to compensate the normal saturation deficit maintained in the attached leaf.

Though the four respiration records are obviously of the same general form, they nevertheless exhibit certain differences which require comment. In the first place, the characteristic transitions back and forth between a higher and lower level of drift are revealed in the record of experiment 1, but they are very poorly developed in the records of the materials grown in the greenhouse. These transitions are more fully described by DUFF and FORWARD (*loc. cit.*). Their significance is still somewhat problematical, but it may be noted here that the occurrence of a transition in the first declining phase of the respiration record promises to complicate the relation between sugar concentration and respiration rate during that phase in which it might be expected to be simplest. Before an R/S relation can be established for these materials, the significance of the transitions must be elucidated.

Other differences to be noted are those between the rates at which CO_2 emission begins in the four records. Before a valid comparison of these initial rates can be made, they must be corrected for differences in the temperature at which the respiration experiments were performed. Using the Van't Hoff-Arrhenius equation the initial rates of experiments 2, 3, and 4 were corrected to the temperature of experiment 1, *i.e.*, to 22.3° C., and the corrected values are brought together in table I.

From this table it is clear that the four initial respiration rates, though of the same order of magnitude, are not equal. The initial rates for experiments 2 and 4 are virtually the same, at 35.9 and 35.5 mg. of CO_2 respectively, but the rate for experiment 3 is 34.0 and for experiment 1 is 37.7 mg. of CO_2. The maximum difference is therefore one of 3.7 mg. This is a significant difference and at first sight might be taken to indicate that the

leaves, at least in experiment 1, were not at a stage of the age sequence strictly comparable to that of the other experimental leaves. Experiments have shown (5) that with plants grown under the uniform artificial conditions maintained in experiment 1, several days are required to produce a change in the initial respiration rate of early mature leaves of 3.7 mg. of CO_2. The difference observed here between the initial rate of experiment 1 and that of experiment 3 is thus the equivalent of a maximum age difference of about three days under the uniform conditions. But since the leaves persist in the "mature" state for better than 20 days, it is clear that a maximum possible difference of 3 days over all is insufficient to call in question their comparability, so far as this can be determined from respiration rates alone.

TABLE I

COMPARISON OF INITIAL RESPIRATION RATES, MINIMAL TRANSITIONAL RESPIRATION RATES AT 22.3° C. AND TOTAL CARBON LOSS IN EXPERIMENTS 1 TO 4

	EXP. NO. 1	EXP. NO. 2	EXP. NO. 3	EXP. NO. 4
	mg.	*mg.*	*mg.*	*mg.*
Initial respiration rate in mg. CO_2 per 100 gm. of leaf fresh weight per hour	37.7	35.9	34.0	35.5
Transitional minimal rate in mg. CO_2 per 100 gm. of leaf fresh weight per hour	24.7	23.7	19.9	22.9
Total carbon loss in mg. CO_2 per 100 gm. of leaf fresh weight per hour	3941	3429	2797	3202

Table I also shows the minimal rates of CO_2 emission to which the high initial rates decline. These have been similarly corrected to the temperature of 22.3° C. so that they are comparable with one another. The values for the minimal rates are evidently correlated with these for initial respiration and also with the total loss of carbon throughout starvation. If the minimal rates are assumed to measure the rate of the CO_2 output of the leaf cells during a brief period in which their physiological state is distinct from preceding and succeeding states, it is evident that this state is characterized by CO_2 output which rises and falls with the pitch of the whole respiration record. On the other hand it will be suggested later that on biochemical grounds the respiration record should be regarded as composite in character, falling into two more or less superimposed parts: (1) the decline from the high initial to low rate; (2) the hump. The correlations just indicated between the observed minimal rates and other measures are of the sort that would be expected were the minimal rates taken to represent transition values between the falling rates of the first declining phase and the rising

rates of the superimposed hump. A consideration of the influence of age upon the form of the respiration record and upon the magnitude of the minimal rates leads to the same conclusion. Where the first declining phase and the hump are well separated in time, as they are in juvenile and senile leaves, the minimal rates are low, and may or may not have a merely transitional significance. But where the two components of the record are considerably superimposed, as with leaves in the mature stage, the minimal rates are high and can hardly have any significance other than that of transitional values (**5**).

The figures for the total CO_2 loss given in table I have not been corrected for the difference in temperature at which experiment 1, on one hand, and experiments 2, 3, and 4, on the other, have been carried out. Experiments by DUFF (**6**) revealed that within the limits of 10° to 30° C., temperature has no significant effect upon the total CO_2 loss.

Changes in the sugar content of the leaves as starvation proceeds are shown for experiments 1 to 4 in figures 6 to 9, respectively. The most

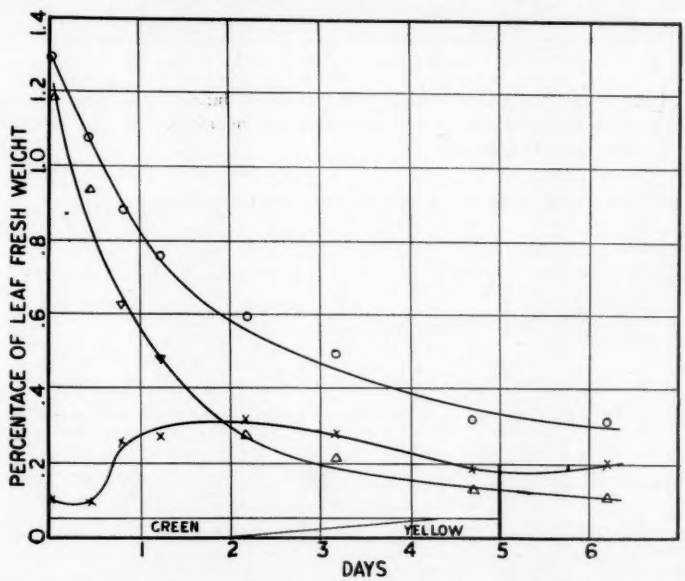

FIG. 6. Sugar content of detached wheat leaves during starvation. ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars (from exp. 1).

striking feature of the four graphs when compared is the variability of the initial sugar content. In this respect experiment 1 is unique since it starts out with a sugar reserve which is several times greater than the highest of

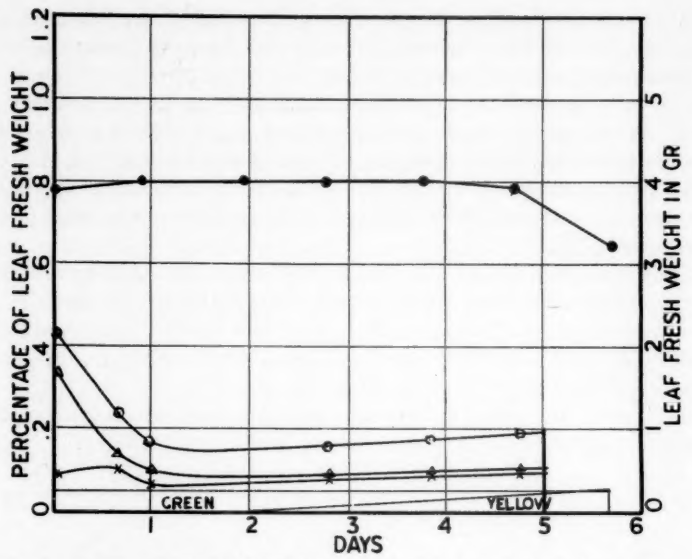

FIG. 7. Sugar content of detached wheat leaves during starvation. ●—●—●, changes in the leaf fresh weight; ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars (from exp. 2).

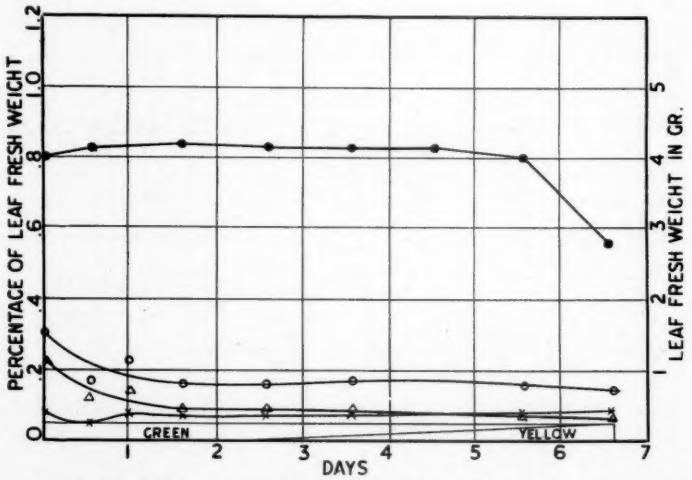

FIG. 8. Sugar content of detached wheat leaves during starvation. ●—●—●, changes in the leaf fresh weight; ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars (from exp. 3).

the others. These are the leaves that were grown under artificial conditions and were permitted 17 hours of continuous assimilation before they were isolated and put in darkness.

In all four experiments the reserve of sugars consists very largely of the non-reducing fraction referred to as "invert sugar," so that gross sugar loss is referable chiefly, if not entirely, to loss of invert sugar. Total sugar and invert sugar fall away in simple declining curves, most rapidly at first and more slowly later. This suggests that the rate of sugar loss might be proportional to the sugar content and this suggestion will be examined

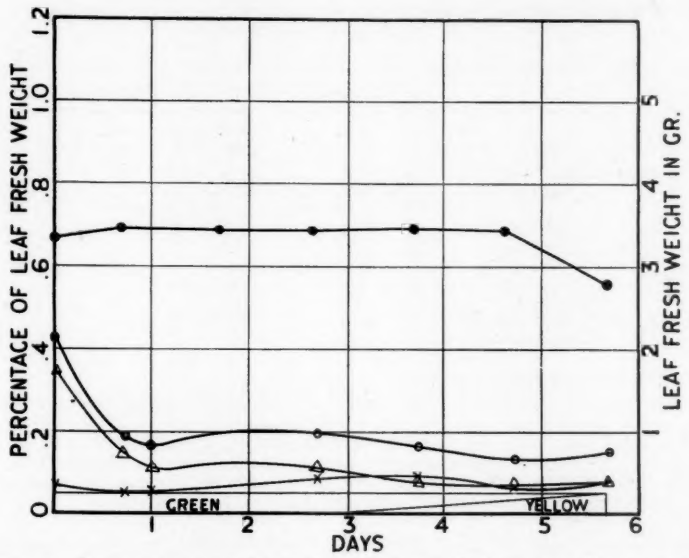

FIG. 9. Sugar content of detached wheat leaves during starvation. ●—●—●, changes in the leaf fresh weight; ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars (from exp. 4).

later in connection with the data from experiment 1. The reducing sugars show an unmistakable increase in the first half of the starvation period in experiment 1, which appears to indicate an excess of inversion over consumption during this period. In the experiments with leaves of low initial sugar content evidences of this increase in reducing sugars are perhaps to be seen but they are poorly developed and uncertain.

When the initial sugar content is low (experiments 2, 3, and 4) sugar loss proceeds in a manner such as to bring the sugar content to more or less steady values long before the end of the starvation sequence. This steady residual sugar value is very nearly the same in all of those experiments

(table II). It is also to be noted that the reducing and non-reducing fractions are present in similar proportions in each case. The residual sugar values are of some theoretical interest and will be considered at a later point of the paper.

Experiment 1 with leaves of high initial sugar content contrasts sharply with the others in respect to sugar depletion. It is obvious that sugar content is still declining at the end of the starvation, the final values are clearly of a higher order of magnitude than that of the residual sugars of experi-

TABLE II

CHANGES IN SUGAR DURING 5 DAYS OF STARVATION, IN MG. PER 100 GR. OF LEAF FRESH WEIGHT

		Exp. no. 1	Exp. no. 2	Exp. no. 3	Exp. no. 4
		mg.	*mg.*	*mg.*	*mg.*
Total sugars	Initial	1291	432	302	424
	Residual	315	170	165	165
	Change in	− 976	− 262	− 137	− 259
Invert sugars	Initial	1187	345	222	360
	Residual	120	92	90	97
	Change in	− 1067	− 253	− 132	− 263
Simple sugars	Initial	104	87	80	64
	Residual	190	78	75	68
	Change in	+ 86	− 9	− 5	+ 4
Residual level is reached in approximately		120 hr.	24 hr.	12–36 hr.*	24 hr.

* In experiment 3, the graph showing variations in the sugar content of leaves within the first 48 hr. could be drawn in several ways, depending on the relative importance attributed to individual sugar values. As a consequence we can conclude that residual values are reached in this experiment sometime between 12 and 36 hours.

ments 2, 3, and 4, and the proportion in which the two fractions occur at the end is very different. The value for residual invert sugar in experiment 1 approaches that of the other experiments, but the reducing sugar fraction is very much higher (table II).

Analysis and interpretation of data

If the form of the respiration record and the totally different form of the graph for total sugar content of the starving leaves are compared it becomes immediately clear that the CO_2 output of the leaf cells cannot be wholly derived from the substances analyzing as sugars on every day of the starvation experience. In experiments 2, 3, and 4 in fact, inspection is sufficient to show that CO_2 production cannot be wholly derived from substances analyzing as sugars on any day. In these three experiments the proportion of the total CO_2 output which is contributed by the sugars is no

more than 11, 7, and 12 per cent. respectively. The leaves of experiment 1 had much the highest initial sugar content, and in them sugars contributed roughly 37 per cent. of the total CO_2. Taking this experiment as an example the CO_2 output and the total sugar loss have been computed (in CO_2 equivalents) for each of the 5 days of starvation and are given in table III.

TABLE III

CARBON DIOXIDE OUTPUT AND TOTAL LOSS OF SUGAR FOR 5 DAYS OF STARVATION

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
CO_2 output, mg. per 100 gm. leaf fr. wt.	689	726	1009	1008	509
Sugar lost in CO_2 equivalents, mg. per 100 gm. leaf fr. wt.	705	279	191	161	95
Sugar as percentage of daily CO_2 output	100%	38%	19%	16%	19%

This table leads to the conclusion that the CO_2 produced on the first day is entirely derived from sugars, but subsequently the contribution of the sugars, both absolute and relative, becomes smaller. Hence it is obvious that some substance or substances not analyzed as sugars are contributing to the CO_2 output in large and increasing proportions after the first day. These substances have not yet been identified and without any implication whatever as to their nature, or to the nature of their degradation products, they will be referred to hereafter as the "secondary substrate materials," in order to distinguish them from the substances analyzed as sugars and which form the "primary substrate materials." The difference between the daily CO_2 production and the daily sugar loss expressed as CO_2 equivalents gives, of course, the daily contribution of this secondary substrate to the CO_2 emission. In experiments 2, 3, and 4 the secondary substrate contributes a substantial proportion of the daily CO_2 even on the first day of starvation.

In figure 10 the respiration record of experiment 1 is reproduced and together with it are plotted the daily mean contributions of the primary and secondary substrates to the CO_2 output. The configuration of these graphs strongly suggests that, as a first approximate formulation, the respiration record should be regarded as a composite one, owing the major peculiarities of its form to the form of its two components, which are:

1. The "primary substrate component," which starts out with high values and declines with time, representing the degradation of the original primary reserve of sugar.
2. The "secondary substrate component," which starts out with low values, rises to a maximum and then drops toward zero, representing the degradation of substances which have not yet been identified.

On this view the minimal values of the respiration record which occur toward the end of the first day must be regarded as transitional, being formed by the declining values of the primary and the rising values of the secondary substrate components. Their magnitude would, therefore, depend upon the form and timing of the two components and should not in these leaves be taken to have any more precise meaning than this.

If then the form of the respiration record is determined in this manner we must proceed to ask on what principles the forms of the two components themselves depend. In order to do this we may regard first the primary

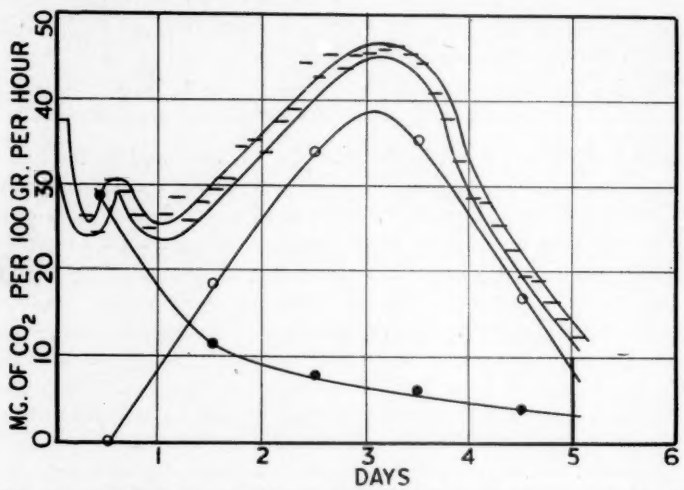

FIG. 10. Contributions of both substrates to CO_2 production on various days of starvation. ═══, respiration record; ●—●—●, primary substrate contribution to CO_2 output; ○—○—○, secondary substrate contribution to CO_2 output (from exp. 1).

component and to begin with, the gross consumption of the primary reserve of sugar over the whole starvation period.

The graphs for total sugar content (figures 7, 8, 9) in experiments 2, 3, and 4 show that the sugars fall off rapidly to residual values which are maintained with but slight evidence of drift or fluctuation (and that not uniform) from the second to the fifth day. In order to get properly weighted average values for the residual values, smooth curves were drawn through the determined points, as in figures 7, 8, and 9, but on a somewhat larger scale of sugar content relative to time. Ordinate values were then taken of points on these graphs separated by intervals of 4 hours throughout the whole period during which the residual values were maintained. The ordinate values were then averaged. The graphs show that the residual

values were much the same in all three experiments and the mean values which are recorded in table II are 170, 165, and 165 mg. for experiments 2, 3, and 4, respectively. The residual value therefore appears to be constant for comparable leaves. It has already been noted that the residual values consist of "invert" and "simple" fractions in roughly constant proportions. From this consideration of the residual values in experiments 2, 3, and 4, it is clear that the total consumption of sugars during the whole starvation period is in this case directly proportional to the initial sugar content.

Another point of interest lies in the fact that in experiments 2, 3, and 4 the residual values are reached long before autolytic disorganization destroys the respiratory system. This means that the final breakdown is not immediately dependent upon exhaustion of the primary reserve of sugar. We must look elsewhere, therefore, for the factor that precipitates the final stage of disorganization which is recognizable by coincident impairment of permeability, exudation, and irregular carbon dioxide output.

Some consideration must now be given to the chemical basis of the residual reducing value and its physiological significance. The residual values are reported as sugar by the analysis and in the discussion so far they have been tacitly assumed to be sugar in the same sense exactly as that larger fraction of the reported sugar which is consumed in the course of starvation. But, obviously, alternative views are possible and must be considered in the light of the effect that their possible confirmation would have upon the conclusions formulated from the present data.

Regarding the residual values first as sugar, four suggestions present themselves:

1. Starvation induces systematic changes, the effect of which is to prevent sugar utilization before the primary sugars are exhausted. This view cannot be maintained because it will be shown later that when the initial sugar content is high, the daily consumption of sugar throughout the whole starvation sequence is roughly proportional to the sugar content.
2. The unaltered respiratory system is subject to a threshold sugar content, an approximate measure of which is given by the residual sugar value. From the kinetic point of view such a state is improbable.
3. The residual values represent an equilibrium concentration of sugars in a system in which sugar is being liberated from combination and utilized in catabolism. The remarkable steadiness of the residual values during a long interval in which respiration rate is at first rising and then falling is difficult to reconcile with this view.
4. The residual values are to be attributed to sugar existing in the tissues in some combination which is broken down during the extraction process. Though no evidence of the reality of this suggestion can yet be put forward, it must be regarded as a serious possibility, if not a probability.

Finally we may note that the residual values might be attributed, partly if not wholly, to substances other than sugar. There is much evidence that such reducing substances occur in extracts of plant tissues (**12, 13**). A curious and constant feature of the residual reducing values, however, is that they are more than doubled after inversion under conditions held to be more or less selective for sucrose. This argues rather for the view that the residual substances are sugars than that they are not and indicates at least that they contain a considerable proportion of sugar.

Evidently the problem of the residual reducing values is obscure. Its solution will probably be found to involve several of the suggestions enumerated above. The fermentation technique, which might be expected to assist in its elucidation, gives anomalous results with extracts of wheat leaves, which indicate the presence of unidentified carbohydrate materials for the fermentation of which the potentialities of the micro-organic reagents are unknown. Much, therefore, must yet be done before a wholly satisfactory account of the residual reducing values can be given. In the meantime, conclusions must be limited to such as will remain valid however this matter turns out. One assumption will be necessary, however, and this is, that among the residual substances of comparable leaves the amount of non-sugar reducers is constant. From what is known of the residual reducing power before and after inversion this assumption appears to be reasonable. In this paper, therefore, the reducing values will continue to be treated altogether as sugar with the reservation that some constant quantity may have to be deducted from the *apparent* to give the *true* sugar content. Such a shift of the base line would entail subsequent numerical modifications, but the principles involved would remain unaffected.

So far the residual value of experiments 2, 3, and 4 alone have been considered and attention must now be directed to experiment 1, which seems to stand alone in this respect. Table II shows that the final low sugar value of experiment 1 is not reached early and maintained for many hours but is reached at the point of disorganization itself. This might mean that in the first experiment the final value is an "apparent residual value" only, and that it represents merely the value that the declining sugar content happens to have attained at the onset of breakdown, and not a value below which starvation will not depress the sugar content, *i.e.*, a value at which there is no apparent sugar consumption. The true residual value of experiment 1 on this view would be smaller than the final value actually achieved. Were the leaves of experiment 1 physiologically comparable to those of the other experiments, we might expect their residual values to be equal. At a later point of the paper an estimate of the true residual value, *i.e.*, the sugar content corresponding to zero sugar loss for experiment 1, will be made. In the meantime it would seem that the apparent gross consumption of reserve

sugar in isolated starving leaves of low to moderate initial sugar content is equal to the initial sugar minus a residual value which is constant for comparable leaves. But at least in leaves of high initial sugar content the apparent consumption may be smaller than that given by this simple rule and there may be a sugar surplus at the time of breakdown.

The form of the primary component of the respiration record also involves a consideration of the question of the change in rate of sugar consumption as starvation proceeds. For this purpose we are confined to the data of experiment 1, since in the other experiments the sugars were depleted too soon to yield a series of successive daily sugar losses.

TABLE IV

DAILY SUGAR CONSUMPTION AND DAILY INITIAL SUGAR CONTENT (EXPERIMENT 1)
(MG. OF SUGARS PER 100 GR. LEAF FRESH WEIGHT)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
Sugar initially present	1291	810	620	490	380
Sugar consumed (observed)	481	190	130	110	65
Sugar consumed (calculated)	459	237	150	91	40

The daily consumption of sugar and the daily initial sugar content for the 5 days of experiment 1 are given in table IV and plotted against each other in figure 11. The distribution of points suggests a linear relation

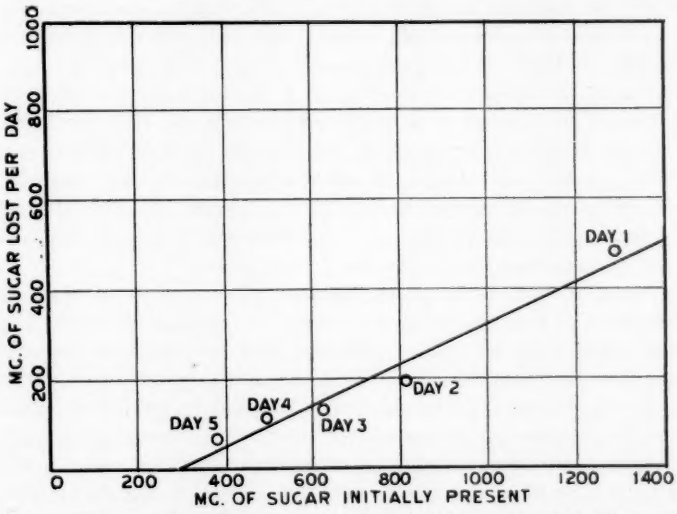

FIG. 11. Daily mean rate of sugar loss and daily initial sugar content (from exp. 1).

within this range of values, and accordingly the constants of the equation of the straight line of best fit have been calculated by the method of least mean squares. The equation is $y = 0.46x - 135$. By use of this equation values for daily sugar consumption have been calculated and in table IV they are compared with those observed. Considering the nature of the primary data, the fit, though far from precise, seems close enough to warrant the conclusion that the relation is rectilinear.

If so, this relation may be formulated in simple terms for experiment 1: 100 gm. of leaves consume each day 135 mg. less than 46 per cent. of the initial sugar content of that day until breakdown occurs. More generally: the daily mean rate of sugar consumption is proportional to the sugar content. This is the principle upon which the form of the primary component of the respiration record is determined. We conclude, therefore, that the first declining phase of the respiration record takes its character from the metabolism of the original primary reserve of sugar, the changing rates of which are a simple function of its diminishing concentration.

This formulation of the data makes it possible to estimate the true residual value for experiment 1. This is obviously given by the intercept of the line $y = 0.46x - 135$ on the axis of the abscissae, since in the other experiments the residual value corresponds to the value of x when $y = 0$. The line intercepts the axis at 293 mg., which is therefore the residual reducing value for experiment 1. This deduced value is so close to the final sugar value reported by analysis (*i.e.*, 315 mg.) that one would hardly be justified in distinguishing between them. It appears, therefore, that the leaves of experiment 1 with their high initial sugar content, became finally disorganized as they approached a point of sugar depletion corresponding to that which the leaves of experiments 2, 3, and 4 entered upon on the second day of the starvation sequence. Evidently two physiological states are represented here. Furthermore, the residual value for experiment 1 is manifestly higher than the corresponding values for the other experiments. This suggests that criteria of comparison in respect to sugar metabolism might possibly be developed from the constants relating daily mean rate of sugar loss to sugar content.

Owing to the low initial sugar content of the leaves in three of the experiments it is not possible to do more here than attempt a merely preliminary examination of this suggestion. For this purpose the available data are assembled in figure 12. We have 5 points for experiment 1, and 2 points for experiments 2 and 4. Data for experiment 2 have not been used for this comparison since, in this experiment as explained earlier in the paper, no definite conclusion could be drawn as to the time when the residual sugar value was reached. The derivation of the line for experiment 1 has been given; that for experiments 2 and 4 seems fairly well

supported by the fact that the corresponding points for the two independent experiments are practically coincident.

This graphic presentation has the effect of comparing the experimental materials on a two-fold basis, the criteria being the magnitude of the residual reducing value (intercept on the axis of x) and the coefficient of proportionality between daily mean rate of sugar loss and sugar content (slope of the line). Evidently experiments 2 and 4 are strictly comparable in both respects and are, in fact, practically identical. These materials were grown in the greenhouse in bright weather; they had low to medium initial sugar content; their low residual values and high slope constant are

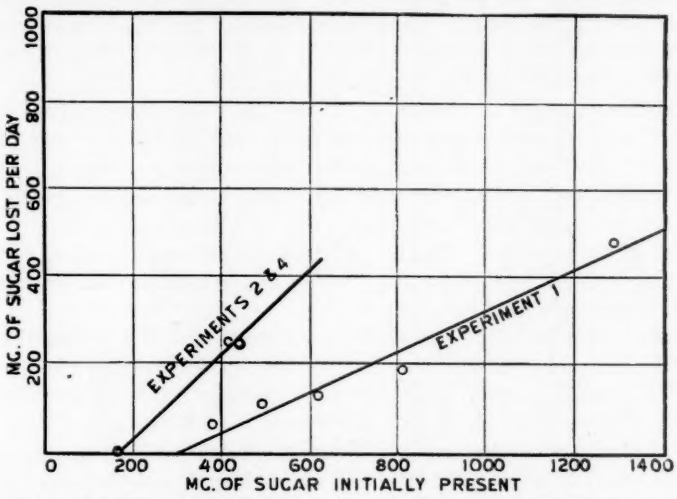

FIG. 12. Relation between sugar content and daily mean rate of sugar loss.

held in common. In experiment 1, the materials for which were grown under steady artificial conditions, we have by far the highest sugar, and a high residual value, but a low coefficient of proportionality. Experiment 1, therefore, stands apart from the others in respect to both criteria.

Before criteria such as these can be properly employed, their physiological significance must be clear. The significance of the residual reducing value has already been discussed. It is not yet clear and may have a purely chemical significance. Should it turn out that the whole of the residual reducing power must be attributed to extraneous interfering substances or the sum of such substances and combined sugar, then all the lines would pass through the origin and this criterion would disappear. Under all other circumstances some real residual sugar must always remain for consideration. The significance of the coefficient of proportionality, on the

other hand, would be a purely physiological one. It would represent the activity of any given sample of leaves at a given temperature in the metabolism of sugar under starvation conditions. Thus two activities appear to be represented in the three experiments. No measurable correlative of activity can as yet be suggested.

We may now turn to the secondary substrate component of the respiration record and review the sequence in time of the changes in its catabolism. As a necessary consequence of the form of the respiration record, the catabolism of the secondary substrate must begin with low daily rates, rising to a maximum roughly coincident with that of the respiration record, then falling toward zero. Without knowledge of the identity of the secondary substrate materials, the analysis cannot be pressed too far, but it seems certain that the liberation of the secondary substrate quota of the CO_2 output involves the degradation of a variety of substances.

The disappearance of chlorophyll at rates corresponding roughly to the rise and fall of the respiration record is easily noted visually. GODWIN and BISHOP (**7**) describe the degradation of the cyanophoric glucoside of the cherry laurel leaf during starvation and find that the changes in the rate of its disappearance are roughly coincident with those of the hump phases of the respiration record. Finally, the work of DELEANO and of YEMM suggests that protein degradation, if examined in relation to the respiration record, might show a similar time relation.

These biochemical changes have their functional correlatives and BLACKMAN (**2**) has described the inactivation, first of the photosynthetic, and finally of the respiratory system in the starving cherry laurel leaf. Clearly, the degradations which accompany the liberation of the secondary substrate quota of the CO_2 output are such as to affect protoplasmic organization. There is little evidence on the extent to which these degradations are coincident or successive, but since the physiological effects are undoubtedly successive and orderly, it is probable that there is a fairly definite order of biochemical events. This unknown order of events determines the manner in which the secondary substrate quota of the CO_2 output is liberated and thus determines the form of the final phases of the respiration record.

Summary

Data are presented on the changes in the rate of CO_2 emission and in the content of simple and complex sugars which proceed in the cells of isolated, first foliage leaves of wheat starving in the dark. The dual form of the highly characteristic changes in the rate of CO_2 emission is shown to have its origin in the degradation of two categories of substances to yield respiratory substrate. The two categories are the original primary reserve of

sugars on the one hand, and on the other, a variety of substances, some of which are of significance in protoplasmic organization. The manner of depletion of reserve sugar controls the course of starvation metabolism in its early stages. The depletion of secondary substrate materials guides the metabolic drift in the later stages and finally causes it to terminate suddenly in autolytic disorganization.

The author wishes to express his thanks to Dr. G. H. DUFF for suggesting this problem, for his help during experimentation, as well as in preparation of the paper. Acknowledgments are due to Professor A. C. NEISH of Queen's University for the use of laboratory space during the summer of 1932, and to the officers of the Bureau of Plant Industry in Washington and the Rust Research Laboratory in Winnipeg for their kindness in supplying the seeds. Part of the experimental expenses in the summer of 1932 was defrayed by a grant from the Science Research Committee of Queen's University.

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DISTRIBUTION OF DIFFERENT NITROGEN FRACTIONS, SUGARS AND OTHER SUBSTANCES IN VARIOUS SECTIONS OF THE PINEAPPLE PLANT GROWN IN SOIL CULTURES AND RECEIVING EITHER AMMONIUM OR NITRATE SALTS*

C. P. Sideris, B. H. Krauss and H. Y. Young

(with eleven figures)

Introduction

Data bearing on the behavior of pineapple plants grown in water cultures and receiving either ammonium or nitrate salts as sources of nitrogen were presented in a former paper (**6**). This present report is concerned with similar information for plants grown in soil- instead of water-cultures. One may be justified in stating that any differences in the behavior between plants of the water- and soil-culture series with comparable nitrogen nutrition are due to chemical changes in the nitrogen of the soil by bacteria. The oxidation of ammonium to nitrate ions in the soil by bacteria, which has played a very important rôle in the present studies, is a well-known fact and needs no discussion.

The literature of plant nutrition with either ammonium or nitrate salts has been reviewed in the former two papers of this series (**5, 6**). For a more thorough and extensive review the papers by Nightingale (**4**) and McKee (**2, 3**) are recommended.

Methods

The plants were grown in two contiguous soil plots, the one designated, for convenience, the ammonium series and the other the nitrate series. Be-

TABLE I

Ammonium nutrient solution

Grams	Name of salt	Grams per liter				
		N	P	K	S	Fe
300	Ammonium sulphate	69.5			72.0	
50	Di-ammonium phosphate	1.1	1.2			
20	Potassium sulphate			9.1	3.6	
25	Iron sulphate				2.8	5.0
	Total	70.6	1.2	9.1	78.4	5.0
Gm. per plant per treatment		3.5	0.06	0.45	4.9	0.25
Gm. per plant per cycle (12 mo.)		28.0	0.48	3.60	39.0	2.00

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ginning when the plants were four months old, *i.e.*, on April 13, 1934, those in each plot were treated, once a month for the subsequent 8 months, with 50 ml. of either an ammonium or a nitrate solution of the compositions as shown in tables I and II.

TABLE II

NITRATE NUTRIENT SOLUTION

GRAMS	NAME OF SALT	GRAMS PER LITER					
		N	P	K	S	Na	Fe
370	Sodium nitrate	61.00				81.00	
6.7	Di-potassium phosphate		1.2	3.1			
15	Potassium sulphate			6.8	2.7		
25	Iron sulphate				3.0		5.0
	Total	61.00	1.2	9.9	5.7	81.00	5.0
Gm. per plant per treatment		3.05	0.06	0.49	0.29	4.00	0.25
Gm. per plant per cycle (12 mo.)		25.00	0.48	3.90	2.30	32.00	2.00

The solution was applied in the axils of mature leaves which, depending on the age of the plants, belonged either to group B or C; that is, to old or mature leaves. At the end of about eleven months, ten plants from each lot were harvested. Their leaves were separated into groups and these, with the stem, into sections according to the plan mentioned in a preceding publication (**6**). A brief description of the sampling and analytical techniques is presented.

The leaves were detached from the stem and placed in groups according to age. The leaves on the basal portion of the stem, having developed earlier than those on the medial and apical portions, were placed in separate groups. Three such groups of leaves were formed: the C-group composed of thoroughly mature leaves; the D-group of active and fully expanded leaves, and the E-group of young leaves only. The stem was also subdivided into sections corresponding to the respective leaf groups attached thereto, and were designated accordingly. Stem sections D and E were combined to furnish sufficient material for analysis. Leaves of the different groups were subdivided into five sections as follows: (1) basal, non-chlorophyllous, in young leaves mostly of meristematic tissues; (2) transitional, subchlorophyllous; (3) lower chlorophyllous; (4) intermediate chlorophyllous; (5) terminal chlorophyllous. The basal section comprised, in all leaf groups, the youngest, and the terminal the oldest tissues of the leaf.

The analytical procedure already discussed (**6**) was adopted, with the exception that fractions of soluble mono-amino nitrogen and basic nitrogen as designated in the former publication were not analyzed separately in these studies but were combined. The nitrogen of both fractions is designated either as rest soluble nitrogen or as combined amino and basic nitrogen.

Experimentation

The various data are presented in tables IV to VIII and in figures 1-A to 9-A and 1-B to 9-B. They are concerned with the weight values of the plants and their various sections, the distribution of moisture, electrical resistance (ohms), titratable acidity and pH, assimilated and non-assimilated nitrogen, total and reducing sugars, and sucrose in the plant tissues.

Plant weights

The weight of the individual plants of both series used for analysis varied considerably. The plants with ammonium nutrition ranged in weight from 3200 to 4500 gm. with a mean value of 3800 gm. while those of the nitrate series ranged from 2400 to 3600 gm. with a mean of 3000 gm. It has not been definitely established whether the difference in mean weight of 800 gm. between the plants of the two series was caused by differences in their nitrogen nutrition or to other causes.

In the protocol below (table III) is reported the mean weight values of the different leaf and stem sections of the two series of plants. The last column shows the ratio values of section weights of the ammonium plants to section weights of the nitrate plants.

TABLE III

Mean weight values of stem and leaf sections in ammonium and nitrate series

Plant sections		Ammonium plants		Nitrate plants		Ratio ammonium/nitrate plants
		Sections	Group	Sections	Group	
		gm.	*gm.*	*gm.*	*gm.*	
Mature leaves C						
Base (white)	1	68		58		
	2	170		188		
	3	257		168		
	4	338		199		
Tip	5	272	1105	147	760	1.45
Active leaves D						
Base (white)	1	145		145		
	2	240		260		
	3	327		203		
	4	390		247		
Tip	5	302	1404	187	1042	1.35
Young leaves E						
Base (white)	1	86		88		
	2	153		153		
	3	126		109		
Tip	4	70	435	65	415	1.05
Stem						
A and B (basal)		229		194		
C to E (apical)		121	350	111	305	1.15

Moisture content of sections

The water content of the different sections of the leaves and stem of the plants of both series was determined for the purpose of comparing the relative succulence of the two lots of plants. Table IV and figures 1-A and 1-B show that the moisture content of the ammonium series was, with a few minor

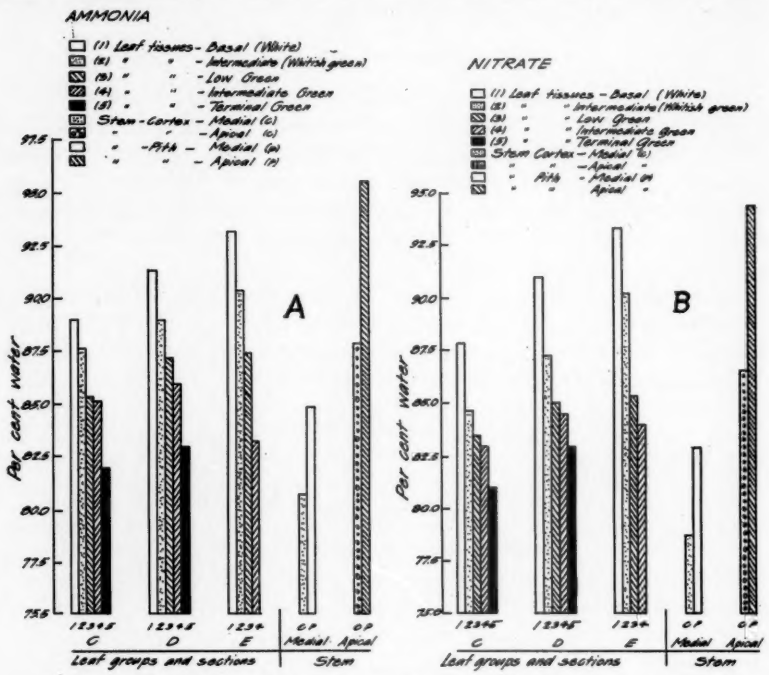

Fig. 1–A. Percentage water in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, in (C) the cortex and (P) pith of the medial and apical sections of the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

Fig. 1–B. Percentage water in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and in (C) the cortex and (P) pith of the medial and apical sections of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

exceptions, greater than that of the nitrate group. The difference in the moisture content of comparable sections is, however, neither very great nor as significant as that found previously when the plants were grown in water cultures. The reason for this condition may be the modified nitrogen nutri-

tion of the plants in soil cultures as the result of oxidation of ammonium ions to nitrate in the soil. The findings are nevertheless in harmony with those obtained from plants grown in the nutrient solution cultures (**6**).

Electrolytes

This is a collective term and comprises all substances of the extracted sap which, when placed between two electrodes in aqueous solution, conduct electric current. The presence of non-electrolytes, such as sugars, etc., interferes to some extent with the free movement of electrolytes and may increase the electrical resistance of the solution.

TABLE IV

Distribution of moisture, electrical resistance, titratable and free acidity of different sections of the leaves and stem of pineapple plants grown in the soil and receiving either ammonium or nitrate nitrogen

Plant sections	Nutrition series							
	Ammonium				Nitrate			
	Water	Resistance	Acidity		Water	Resistance	Acidity	
			Citric acid	pH			Citric acid	pH
Leaves	%	*ohms*	%		%	*ohms*	%	
Mature								
C1 (Base)	89.0	89	0.03	5.0	87.8	124	0.03	5.0
C2	87.7	58	0.03	5.0	84.6	64	0.19	4.6
C3	85.3	51	0.45	4.6	83.0	51	0.96	4.4
C4	85.2	48	0.57	4.5	83.0	44	1.82	4.0
C5 (Tip)	82.0	44	1.75	3.8	81.4	44	2.24	3.8
Active								
D1 (Base)	91.4	71	0.05	5.2	91.0	90	0.10	5.0
D2	89.0	80	0.03	5.0	.....	83	0.22	4.6
D3	87.2	81	0.48	4.2	85.0	83	0.70	4.2
D4	86.0	60	1.40	3.8	84.5	68	1.60	3.7
D5 (Tip)	83.0	53	1.45	3.6	83.0	44	2.24	3.5
Young								
E1 (Base)	93.2	66	0.05	5.2	93.4	79	0.13	5.2
E2	90.3	102	0.07	5.0	90.2	114	0.16	5.0
E3	87.4	77	1.00	3.7	85.4	95	0.64	4.0
E4 (Tip)	83.2	71	1.35	3.8	84.0	75	1.40	3.6
Stem								
Medial								
Cortex	80.7	50	0.13	5.0	.....	50	0.10	5.0
Pith	84.8	41	0.16	5.0	.....	48	0.16	5.0
Apical								
Cortex	87.8	63	0.23	5.2	.....	69	0.19	5.0
Pith	95.6	47	0.26	5.0	.....	53	0.26	5.0

The data as presented in table IV and figures 2-A and 2-B show that there is a trend of electrolyte accumulation in the mature terminal sections of the leaves, while the young white basal and intermediate sections contain corre-

spondingly smaller quantities. It will be observed that the sections of the youngest leaves, *i.e.*, E leaves of both plants supplied with ammonium and with nitrate, contain considerably smaller amounts of electrolytes. This condition occurring more frequently in the youngest leaves of the plant, may be associated with certain physiological changes in the tissues undergoing rapid differentiation. Evidence obtained elsewhere on the distribution of

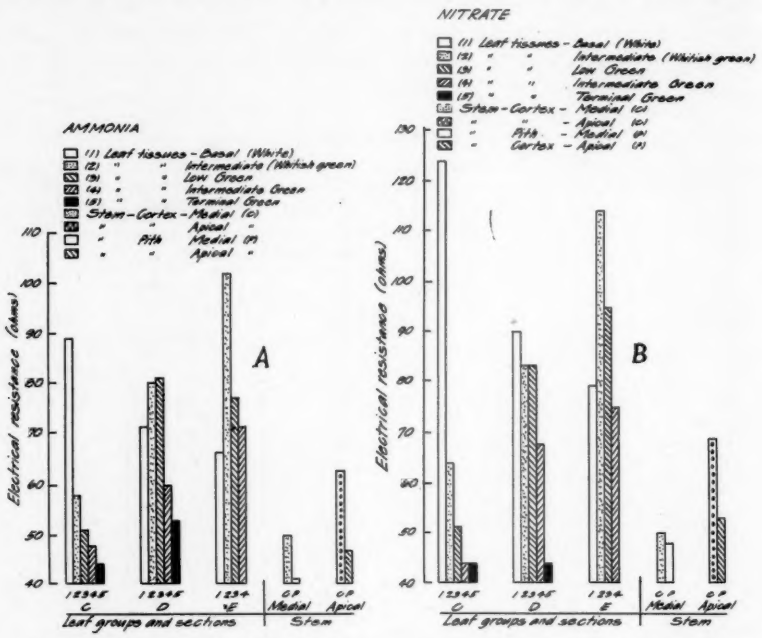

FIG. 2–A. Electrical resistance (ohms) of the extracted sap of (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and of (C) the cortex and (P) pith of the medial and apical sections of the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

FIG. 2–B. Electrical resistance (ohms) of the extracted sap of (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, (E) young leaves, of (C) the cortex, and (P) pith of the medial and apical sections of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

certain forms of soluble organic nitrogen and carbohydrates in the various sections of the E group of young leaves is in harmony with this hypothesis. The electrolytes ordinarily present in the extracted plant sap are potassium, nitrate, amino acids, and various hydroxy-acids. Calcium and magnesium

salts, amides, etc., occur in comparatively smaller amounts than the above mentioned substances. Detailed accounts on the distribution of different electrolytes in different parts of the plant will be presented in a forthcoming publication.

The data show, in general, that electrolytes are present in greater amounts in the older than in the younger groups of leaves and in greater amounts in the older tissues of any one group of leaves.

Acidity

Titratable acidity and pH were determined on the extracted sap and are presented in table IV. The former values are expressed as per cent. citric acid, although other organic acids besides citric normally occur in the tissues.

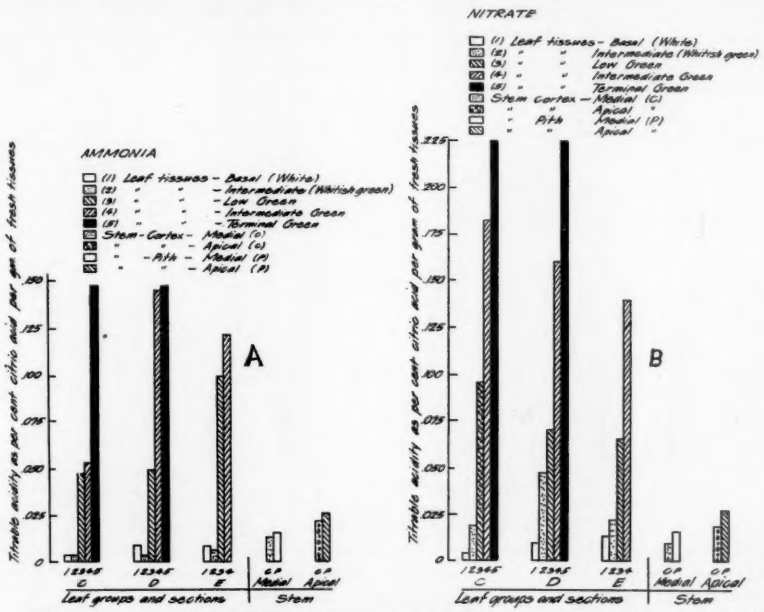
AMMONIA
(1) Leaf tissues – Basal (White)
(2) " " – Intermediate (Whitish green)
(3) " " – Low Green
(4) " " – Intermediate Green
(5) " " – Terminal Green
Stem – Cortex – Medial (C)
" " – Apical (C)
" – Pith – Medial (P)
" " – Apical (P)
Titrable acidity as per cent citric acid per gm of fresh tissues
A
Leaf groups and sections
Stem
Medial
Apical
NITRATE
B

Fig. 3–A. Titratable acidity as per cent. citric acid of the extracted sap (1) of the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

Fig. 3–B. Titratable acidity as percentage of citric acid of the extracted sap of (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

The pH values were obtained colorimetrically, after dilution of the sap with water and proper masking of inherent colors and turbidity. Figures 3-A and 4-A present the distribution of titratable and free acidity (pH) in the various sections of the leaves and stem of the plants receiving ammonium nutrition; figures 3-B and 4-B present similar data for corresponding sections of the plants supplied with nitrate.

There are no appreciable differences in the pH values of corresponding plant sections of the two different lots of plants. Those that may be observed are in most cases within the range of experimental errors. The amounts of total acids were greater for the nitrate than for the ammonium lot of plants. The differences are comparatively great and significant. The causes for the difference in the acidity of corresponding sections of the two different lots of plants cannot be explained without additional experimental evidence. CLARK (1) working with tomato plants grown respectively in ammonium and nitrate solution cultures obtained similar results.

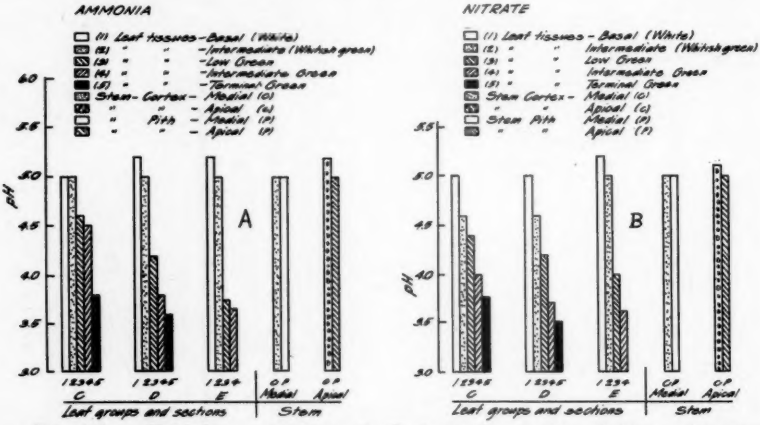

FIG. 4–A. pH of the extracted sap of (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

FIG. 4–B. pH of the extracted sap of (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

DISTRIBUTION OF NITROGENOUS FRACTIONS

The total nitrogen content of the different sections of the leaves and stem of both lots of plants was divided into non-assimilated, or inorganic nitrogen, and into assimilated, or organic nitrogen. The former consisted mostly of

TABLE V

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, AND INSOLUBLE ORGANIC NITROGEN FRACTIONS PER GRAM OF FRESH WEIGHT IN DIFFERENT SECTIONS OF THE LEAVES AND STEM OF *Ananas comosus* (L.) MERR. GROWN IN TWO DIFFERENT SOIL PLOTS AND RECEIVING MONTHLY APPLICATIONS OF EITHER AMMONIUM OF NITRATE NITROGEN

Plant sections	Nutrition series																		
	Ammonium-nitrogen									Nitrate-nitrogen									
	Inorganic		Organic							Inorganic		Organic							
			Soluble			Insoluble						Soluble			Insoluble				
	NH_4	NO_3	Glutamine	Asparagine	Rest	Amide	Monoamino	Basic	Humin	NH_4	NO_3	Glutamine	Asparagine	Rest	Amide	Monoamino	Basic	Humin	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Leaves																			
Mature																			
C1 (Base)	0.014	0.488	0.075	0.089	0.504	0.062	0.358	0.084	0.067	0.029	0.702	0.043	0.070	0.734	0.088	.....	0.084	0.087	
C2	0.010	0.506	0.070	0.046	0.514	0.062	0.297	0.112	0.084	0.010	0.514	0.047	0.069	0.734	0.133	.....	0.193	0.140	
C3	0.009	0.178	0.088	0.072	0.480	0.081	0.264	0.220	0.178	0.010	0.092	0.048	0.082	0.612	0.231	.....	0.480	0.252	
C4	0.010	0.076	0.082	0.101	0.642	0.136	1.080	0.480	0.250	0.010	0.034	0.048	0.075	0.528	0.215	.....	0.660	0.266	
C5 (Tip)	0.009	0.068	0.081	0.128	0.514	0.154	1.080	0.424	0.202	0.016	0.034	0.041	0.082	0.442	0.231	.....	0.575	0.296	
Active																			
D1 (Base)	0.013	0.460	0.090	0.150	0.750	0.067	0.364	0.113	0.055	0.027	0.474	0.038	0.115	0.824	0.098	0.350	0.119	0.059	
D2	0.008	0.383	0.054	0.059	0.468	0.059	0.258	0.103	0.073	0.018	0.400	0.050	0.058	0.493	0.090	0.310	0.200	0.053	
D3	0.011	0.153	0.064	0.039	0.382	0.086	0.432	0.168	0.120	0.031	0.032	0.050	0.070	0.403	0.158	0.414	0.416	0.174	
D4	0.012	0.058	0.071	0.075	0.628	0.120	0.828	0.336	0.230	0.030	0.036	0.032	0.055	0.259	0.160	0.932	0.460	0.207	
D5 (Tip)	0.010	0.058	0.076	0.086	0.605	0.154	1.230	0.316	0.260	0.035	0.039	0.038	0.065	0.259	0.130	0.930	0.500	0.238	
Young																			
E1 (Base)	0.012	0.224	0.084	0.104	0.650	0.080	0.750	0.154	0.080	0.040	0.224	0.040	0.120	0.768	0.100	0.530	0.166	0.067	
E2	0.009	0.175	0.062	0.062	0.403	0.070	0.415	0.096	0.084	0.023	0.154	0.032	0.054	0.365	0.073	0.490	0.132	0.090	
E3	0.009	0.070	0.061	0.058	0.258	0.092	0.684	0.185	0.141	0.010	0.043	0.050	0.081	0.235	0.113	0.790	0.292	0.151	
E4 (Tip)	0.010	0.029	0.071	0.069	0.275	0.133	1.010	0.264	0.214	0.017	0.032	0.071	0.086	0.235	0.148	0.890	0.440	0.151	
Stem																			
Medial																			
Cortex B	0.043	0.383	0.099	0.159	1.230	0.129	0.448	0.176	0.157	0.040	0.974	0.055	0.185	1.430	0.101	0.592	0.196	0.150	
Pith B	0.052	0.440	0.091	0.190	1.560	0.095	0.414	0.170	0.107	0.035	1.064	0.055	0.226	1.720	0.095	0.560	0.198	0.107	
Apical																			
Cortex C	0.036	0.266	0.128	0.200	1.170	0.105	0.623	0.126	0.100	0.070	0.648	0.089	0.290	1.075	0.115	0.666	0.246	0.104	
Pith C	0.045	0.355	0.168	0.328	1.500	0.116	0.584	0.168	0.098	0.032	0.954	0.087	0.376	1.290	0.125	0.643	0.180	0.098	

nitrate nitrogen which was absorbed by the roots directly from the soil, and of ammonium nitrogen which may represent either non-assimilated nitrogen or nitrogen derived from the proteins of senile tissues by enzymatic hydrolysis, or that resulting through reduction of nitrate. The organic or assimilated nitrogen was divided into soluble and insoluble fractions. The soluble fraction of the organic nitrogen was segregated into glutamine, asparagine, and rest soluble (amino plus basic) nitrogen. The insoluble portion was fractionated, after hydrolysis with 20 per cent. sulphuric acid, into amide, amino and basic nitrogen, and humin nitrogen.

The distribution of these nitrogeneous fractions in the different sections of the leaves and stem may be seen in tables V to VII and in figures 5 to 8.

The data presented below comprise values obtained for leaf and stem tissues but not for roots. The great difficulty encountered in extricating roots from the soil has prevented us from obtaining reliable samples for these studies.

Stem

Only the medial and apical sections of the stem, to which leaf groups C, D, and E were attached, were reserved for chemical analysis. The basal section was discarded on account of the senile condition of the tissues. The cortex was separated, as carefully as possible, from the pith before analysis. The results obtained with the two different lots of plants were as follows:

Ammonium series.—The analytical data presented in figure 5-A show that the amounts of ammonium nitrogen in the various stem sections were very small as compared with those of nitrate nitrogen, in spite of the fact that the plants were supplied exclusively with ammonium salts. The amounts of both ammonium and nitrate were greater in the pith than in the cortex, possibly because of the preponderance of water-conducting elements in the former tissue. The small amounts of ammonium can be explained either by the assumption of its conversion to nitrate in the soil before absorption, or by its rapid assimilation, as was found in our former studies (**6**). It is possible that both processes operated simultaneously.

The values for the various fractions of soluble organic nitrogen were comparatively high throughout the plant. The combined values of amino and basic nitrogen especially appear very high when they are contrasted with the amide fractions (glutamine plus asparagine). The values of all such fractions, with a single minor exception, were consistently greater for the pith than for the cortex. The values for the different fractions of insoluble

Fig. 5–A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in the cortex and pith of the medial and apical sections of the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

Fig. 5–B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in the cortex and pith of the medial and apical sections of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

A
B
mg. per gram of fresh tissues
AMMONIA
NITRATE
Inorganic
Soluble organic
Insoluble organic
NITROGEN

organic, or protein nitrogen, however, were usually greater for the cortex than for the pith. The greater amounts of protein and smaller amounts of soluble organic nitrogen present in the cortex indicate a greater rate of conversion of the soluble organic nitrogen to protein in the cortex than the pith. The formation of greater amounts of protein in the cortex were favored by the presence of relatively larger quantities of sugars.

NITRATE SERIES.—The data in figure 5-B and table V show that the amounts of ammonium in the different sections of the stem were very small and these amounts may represent nitrogen which had been derived from proteins by enzymatic hydrolysis or through nitrate reduction. The amounts of nitrate, however, were very great and represent nitrogen in its native state, *i.e.*, as it was absorbed by the roots from the soil and before its conversion to one of its many organic forms. The amounts of nitrate were greater in the pith, possibly for the same reasons already mentioned. The soluble organic nitrogen content, particularly the combined fractions of amino and basic nitrogen, was very high. The amounts of asparagine were slightly greater in the plants supplied with nitrate than in those supplied with ammonium, whereas the opposite condition was true with glutamine.

Protein was present in moderate amounts, the values comparing favorably with those of the preceding series.

MATURE LEAVES (C-GROUP)

AMMONIUM SERIES.—The leaves belonging to this group are the first ones produced by the propagating material after planting, as discussed in a preceding publication (**6**). At the time of harvest they were thoroughly mature and a few of them had become senile. Figure 6-A, containing the results of the chemical analyses of the different sections, shows that ammonium was present only in traces, while nitrate occurred in great amounts in the non-chlorophylllous and sub-chlorophyllous sections (C_1 and C_2). In the chlorophyllous sections the quantities of nitrate dropped very suddenly to low values. Soluble organic nitrogen values were intermediate and approximately of the same magnitude as those found in other studies (**6**). The fluctuations in the different nitrogenous fractions of the various sections cannot be satisfactorily explained in all cases. Protein values were comparatively high, indicating that conditions for its synthesis were favorable.

NITRATE SERIES.—Figure 6-B, illustrating the results of the chemical analyses of the different leaf sections, shows that the amounts of ammonium were very small, while those of nitrate were very great. Nitrate was restricted to the basal non-chlorophyllous sections, whereas ammonium, although occurring in very small amounts, was distributed in approximately uniform amounts in the different sections. The nitrate values for the non-chlorophyllous sections were considerably higher in this lot of plants than in the

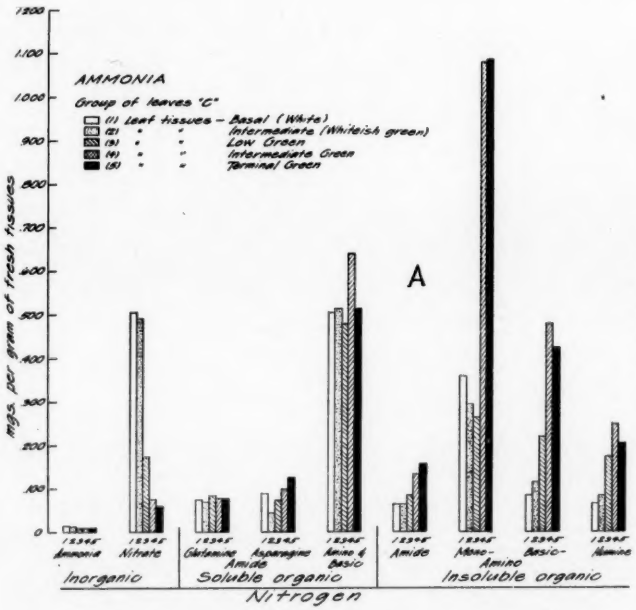

FIG. 6–A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature group of leaves of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

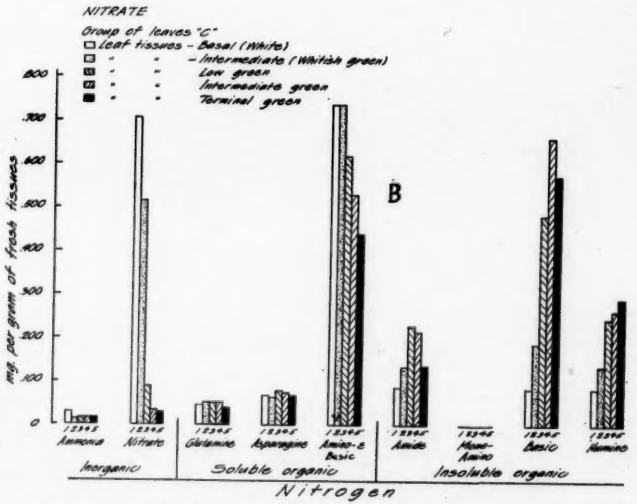

FIG. 6–B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature group of leaves of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

preceding one, indicating that applications of nitrate salts increased the nitrate content of plant tissues more rapidly than did the application of ammonium salts. Ammonium may be absorbed by the roots either as ammonium, or as nitrate, after oxidation to this latter form. When absorbed in the former state, only the values for the organic nitrogen fractions rise to high levels, while those for inorganic nitrogen, and especially for ammonium, remain very low. The amounts of the combined amino and basic fractions of soluble nitrogen were appreciably greater in the basal than in the intermediate and terminal sections of the leaves. The amounts of the two amide fractions (glutamine and asparagine) of soluble nitrogen fluctuated within a very narrow range in the different sections. The values for practically all the fractions of protein were low in the basal but high in the intermediate and terminal sections of the leaves. When the high figures for soluble organic nitrogen and low figures for protein in the basal sections are contrasted with the low content of soluble organic nitrogen and the high concentration of protein of the intermediate and terminal sections, they show that, as the values for soluble organic nitrogen decrease, those for protein increase. The low values for basic nitrogen in the C_5 section (tip of old leaves) have possibly been obtained as a result of hydrolytic processes, which often develop in senile tissues.

Active leaves (D-group)

Ammonium series.—The D-group is composed of the longest, fully expanded and possibly most active leaves of the entire plant (**6**). The analytical data as presented in figure 7-A show that the amounts of ammonium were very small in all plant sections. The amounts of ammonium nitrogen which occur ordinarily in pineapple plant tissues are small regardless of the form of inorganic nitrogen applied to the plants. The ammonium found probably represents quantities derived from hydrolyzed native protein or from nitrate reduction. The amounts of nitrate were very high in the non-chlorophyllous basal sections but considerably smaller in the intermediate- and terminal-chlorophyllous sections. This condition is characteristic of pineapple plants absorbing nitrate from a substratum.

Soluble organic nitrogen was found in greater quantities in the non-chlorophyllous basal than in the intermediate-chlorophyllous section of the leaves. The quantities in the mature terminal sections of the leaves rose to considerably greater values than in the intermediate ones, possibly either on account of retarded protein synthesis or of protein hydrolysis in the relatively senile tissues concerned.

With respect to the distribution of protein nitrogen, the amounts of different fractions of this form of nitrogen were low in the meristematic white section but high in the terminal-chlorophyllous sections. The values

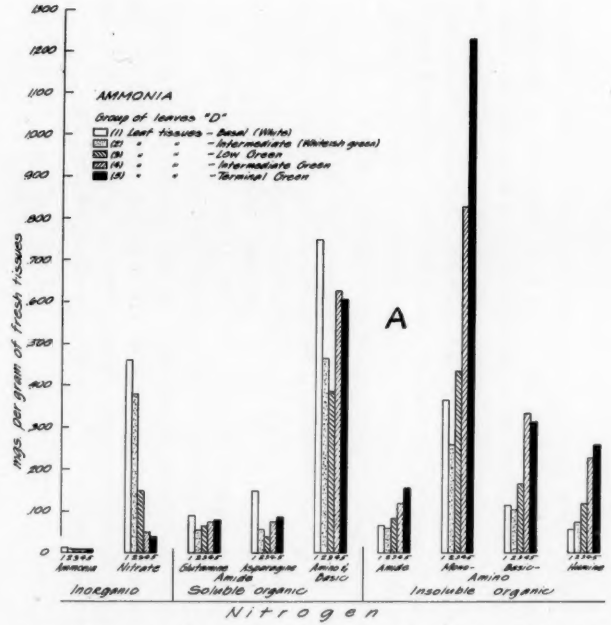

FIG. 7–A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (D) the active group of leaves of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

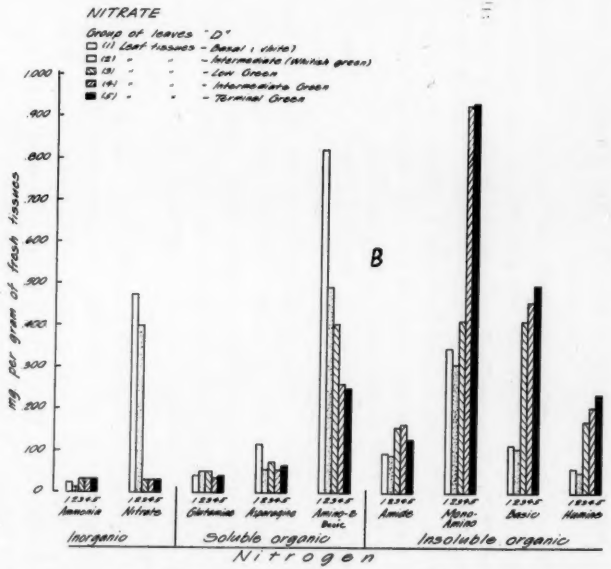

FIG. 7–B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (D) the active group of leaves of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

obtained harmonize well with those of the older leaf group C of the ammonium series.

NITRATE SERIES.—The amounts in grams of inorganic nitrogen as presented in figure 7-B show low values for ammonium and high values for nitrate. The values for ammonium were found to fluctuate in the different sections between 0.018 and 0.035 mg. while those for nitrate were between 0.032 and 0.474 mg. The high values for nitrate nitrogen were always associated with the non-chlorophyllous and the low with the chlorophyllous sections, as has been found in former cases. The combined fractions of amino and basic nitrogen were very high in the non-chlorophyllous basal sections and comparatively low in the chlorophyllous terminal ones. This condition did not develop in the same group of leaves in the ammonium series. In this series the values for amino and basic nitrogen decreased in the intermediate sections but increased in the terminal ones. Asparagine nitrogen was higher in the non-chlorophyllous than in the chlorophyllous sections, a condition which was also obtained in the corresponding group of leaves in the ammonium series.

The values for fractions of protein nitrogen were low in the non-chlorophyllous basal sections. In the intermediate- and terminal-chlorophyllous sections they rose gradually to high levels. The mono-amino fractions of protein nitrogen were found in nearly all cases to be lower in the plants supplied with nitrate than in plants supplied with ammonium salts, while the opposite was true with the basic fraction.

YOUNG LEAVES (E-GROUP)

AMMONIUM SERIES.—The E-group is composed of the youngest and most actively growing leaves of the plant (**6**). The analytical data presented in figure 8-A show that the quantities of ammonium were very low in all sections. Those of nitrate of the non-chlorophyllous sections, although high, are nevertheless considerably lower than in corresponding sections of the mature D and older C groups of leaves. This condition has been attributed (**6**) to an incomplete development of the fibrovascular bundles in the young apical section of the stem whereon the bases of these leaves are attached.

The amounts of the combined fractions of amino and basic soluble nitrogen were high in the non-chlorophyllous basal sections but low in the chlorophyllous terminal ones. The values for asparagine nitrogen are of the same order as those for the fraction of amino and basic nitrogen. The small amounts of the soluble organic nitrogen fractions in the chlorophyllous sections probably indicate a condition of rapid protein synthesis.

The amounts of the different fractions of protein nitrogen were slightly lower in the intermediate than in the basal and terminal sections. Humin nitrogen, regardless of leaf group or original source of nitrogen supply, was lowest in the basal and highest in the terminal sections of the leaves.

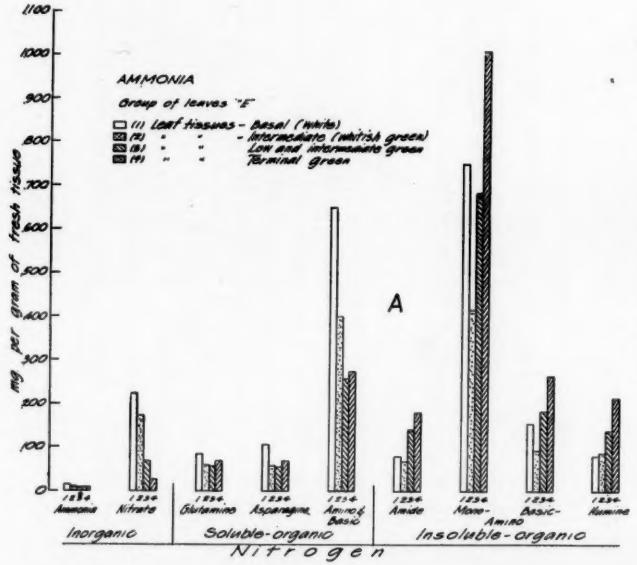

FIG. 8–A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (E) the young group of leaves of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

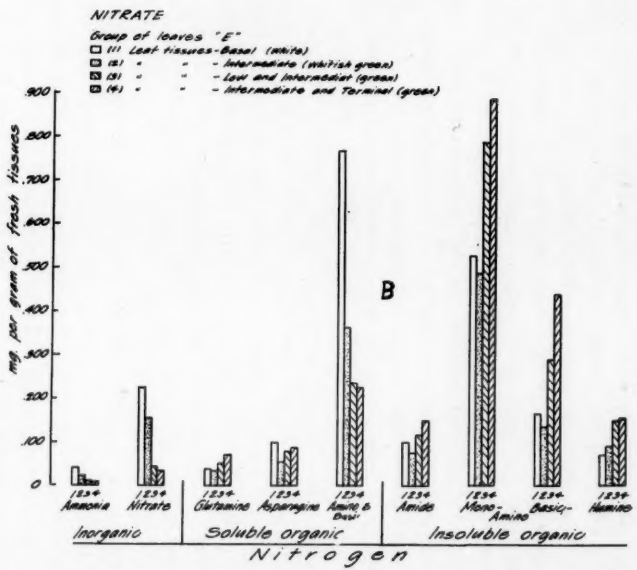

FIG. 8–B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (E) the young group of leaves of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

NITRATE SERIES.—Figure 8-B, presenting the analytical results for the various leaf sections of the nitrate series, shows that the amounts of ammonium nitrogen were low, while those of nitrate nitrogen were comparatively high. The quantities of nitrate in the basal sections of the leaves of this group were considerably less than in the same sections of the former two leaf groups. An explanation of this condition was offered above. The values are of the same magnitude as those for the corresponding leaf group of the ammonium series. The consistently higher values for ammonium in the various sections of the leaves from plants receiving nitrate as compared with those for sections of leaves from plants receiving ammonium nutrition cannot be explained other than on the assumption that they either represent reduced, but not assimilated nitrate, or hydrolyzed protein. The amounts of the combined fractions of amino and basic soluble nitrogen were higher in the non-chlorophyllous basal sections than in the chlorophyllous ones, a condition which is quite similar to the one observed in the plants receiving ammonium nutrition. The values of the two fractions of amide nitrogen (glutamine and asparagine) also had approximately the same order of distribution as they did in the other lot of plants.

The different fractions of protein were present in expected quantities, *i.e.*, their values were moderate in the basal, high in the terminal, and low in the intermediate sections. The humin nitrogen fraction was an exception and was present in quantities similar to those mentioned in the description of the preceding series. The basic nitrogen fraction of protein was higher in the nitrate than in the ammonium series of plants.

DISTRIBUTION OF SUGARS

The analytical data presented in table VI and figures 9-A and 9-B show the distribution of total, reducing sugars, and sucrose, the latter representing the difference between the two former values. No other kinds of carbohydrates were determined on account of accidental spoilage of the dried tissues.

AMMONIUM SERIES.—Figure 9-A, representing the amounts of the above mentioned sugars found in the different sections of the ammonium lot of plants, shows that the terminal sections contained, in general, more sugars than the intermediate ones. The basal sections in well developed and mature leaves contained greater amounts of sugars than some of the intermediate sections in the same group. In young leaves, however, the amounts of sugars in the basal sections were relatively low, presumably because they were used in tissue building. The accumulation of sugars in the basal sections of well grown and mature leaves is the result of either a decreased rate in the processes of building new tissue or a result of impeded translocation; or, a result of both of these acting simultaneously. The amounts of sugars

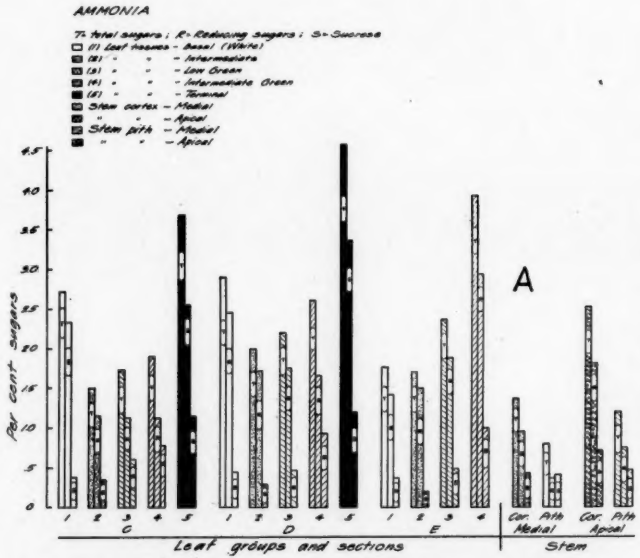

FIG. 9–A. Distribution of reducing and total sugars and sucrose in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and in (C) the cortex and (P) pith of the medial and apical sections of the stem of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

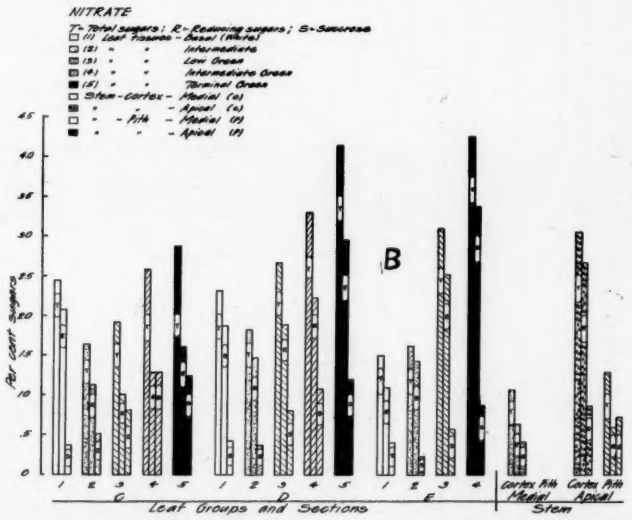

FIG. 9–B. Distribution of reducing and total sugars and sucrose in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and in (C) the cortex and (P) pith of the medial and apical sections of the stem of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

TABLE VI

DISTRIBUTION OF TOTAL SUGARS, REDUCING SUGARS, AND SUCROSE IN DIFFERENT SECTIONS OF THE LEAVES AND STEM OF PINEAPPLE PLANTS GROWN IN THE SOIL AND RECEIVING EITHER AMMONIUM OR NITRATE NITROGEN

PLANT SECTIONS	NUTRITION SERIES					
	AMMONIA			NITRATE		
	SUGARS			SUGARS		
	TOTAL	REDUCING SUGARS	SUCROSE	TOTAL	REDUCING SUGARS	SUCROSE
	%	%	%	%	%	%
Leaves						
Mature						
C1 (Base)	2.73	2.34	0.39	2.45	2.08	0.37
C2	1.49	1.15	0.34	1.64	1.13	0.51
C3	1.72	1.12	0.60	1.92	1.01	0.81
C4	1.91	1.14	0.77	2.58	1.29	1.29
C5 (Tip)	3.67	2.54	1.13	2.86	1.61	1.25
Active						
D1 (Base)	2.91	2.47	0.44	2.31	1.88	0.43
D2	2.00	1.71	0.29	1.83	1.47	0.36
D3	2.21	1.75	0.46	2.66	1.87	0.79
D4	2.61	1.67	0.94	3.30	2.22	1.08
D5 (Tip)	4.56	3.36	1.20	4.14	2.94	1.20
Young						
E1 (Base)	1.78	1.42	0.36	1.49	1.09	0.40
E2	1.70	1.50	0.20	1.63	1.41	0.22
E3	2.36	1.88	0.48	3.10	2.52	0.58
E4 (Tip)	3.93	2.94	0.99	4.25	3.37	0.88
Stem						
Medial						
Cortex	1.37	0.94	0.43	1.06	0.64	0.42
Pith	0.80	0.37	0.43	.....	.....	.....
Apical						
Cortex	2.52	1.80	0.72	3.05	2.17	0.88
Pith	1.22	0.75	0.47	1.31	0.59	0.72

were greater in the sections of the D-group of leaves than in those in either the C- or E-groups, indicating that protoplasmic vigor and a retarded rate of the processes of building new tissue were responsible for the greater amounts of sugars. Although protoplasmic vigor was as great and possibly greater in the cells of the young E-group of leaves, the smaller amounts of sugars present were due to a very great rate of synthesis of new tissues. Partial lack of protoplasmic vigor may be the cause of the relatively smaller amounts of sugars found in the sections of the oldest, or C-group, as compared to those found in the fully expanded but very active D-group of leaves. The order of distribution of sucrose was about the same in all three groups of leaves. The amounts in the terminal sections were from 2 to 3 times greater than in the basal ones, section 2 having the lowest value in all leaf groups.

The amounts of sugars in the cortex and pith of the stem were very

unequally distributed. The cortex contained two and one-half times more reducing sugars than the pith. The greater amounts of sugars in the cortex were expected because of the direct connection of this tissue with the leaf bases. The smaller amounts of sugars in the pith were possibly attributable either to a rapid conversion of substances to starch, or to a decreased rate of translocation. The young apical section of the stem contained greater amounts of sugars than the medial section, possibly because of the attachment to it of leaf groups D and E, which are more efficient sugar producers as a result of their greater protoplasmic vigor, than leaves of group C which are relatively senescent.

NITRATE SERIES.—The order of distribution of sugars in the different sections of the plants of the nitrate series was about the same as in the ammonium group. There were, in some cases, slight differences but none were sufficiently great to be of significance. The comments presented in the preceding case apply equally well to this one.

Discussion

The analytical data presented in the foregoing studies have indicated that the chemical composition of plants grown in non-sterilized soil cultures and supplied with either ammonium or nitrate salts were, with a few exceptions, about the same. The exceptions were in the values for the water content of the tissues which were slightly greater for the ammonium than for the nitrate lot of plants. One may state also, by analogy, that the amounts of organic matter per gram of fresh weight were smaller in the ammonium than in the nitrate lot of plants. Regardless of how this condition is stated, the facts are that, with ammonium nutrition, the percentage of organic matter produced per unit of fresh weight is considerably smaller than with nitrate nutrition. Some differences found in the electrical resistance of the extracted sap of corresponding leaf and stem sections of the two lots of plants were slightly greater in the nitrate than in the ammonium series. The titratable acidity of the expressed sap was considerably greater in the plants of the nitrate than in those of the ammonium series. The greater moisture content of the plants of the ammonium series has been discussed in certain other studies (**6**). With respect to the greater values of electrical resistance, no satisfactory explanation can be offered until complete data are obtained on the chemical composition of the sap. The greater acidity values in the plants of the nitrate series cannot be explained without additional experimental data.

The distribution of the different nitrogen fractions in the various sections of the leaves and stem of the two lots of plants was, with minor exceptions, about the same. In our former studies (**6**), where plants were grown in solution cultures instead of soil, the chemical composition of the plants

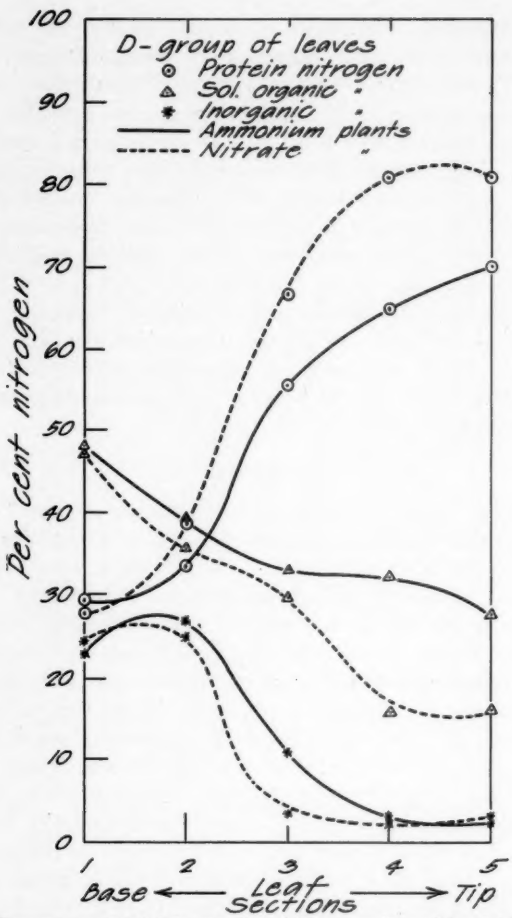

FIG. 10. Percentage of total inorganic, soluble organic, and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (D) the active group of leaves of plants grown in soil and supplied either with an ammonium or with a nitrate salt as the source of nitrogen.

receiving ammonium nitrogen differed considerably from that of the plants supplied with nitrate. Such differences have been slight in the two lots of plants grown in soil because of the rapid oxidation in the soil of ammonium to nitrate and the subsequent simultaneous absorption of both ions by the plants. The presence of nitrate in the non-chlorophyllous basal sections of the leaves and in the stem of the plants receiving ammonium salts is ample

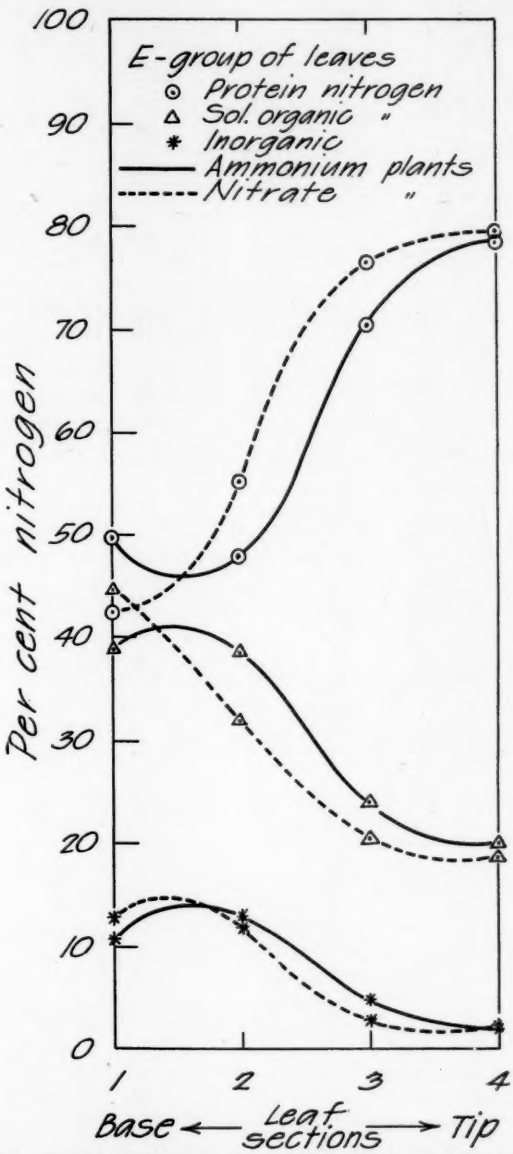

FIG. 11. Percentage of total inorganic, soluble organic, and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (E) the young group of leaves of plants grown in soil and supplied either with an ammonium or with a nitrate salt as the source of nitrogen.

TABLE VII

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT SECTIONS TOGETHER WITH PERCENTAGE VALUES OF THE SAME AND OF THEIR FRACTIONS AS FOUND IN PINEAPPLE PLANTS GROWN IN SOIL CULTURES RECEIVING AMMONIUM NITROGEN

Plant sections	Inorganic				Soluble organic					Insoluble organic						Total organic N	Total N
	Percentage of inorganic		Total	Percentage of total	Percentage of soluble organic			Total	Percentage of total	Percentage of insoluble organic				Total	Percentage of total		
	NH_4	NO_3			Glutamine	Asparagine	Rest N			Amide N	Mono-amino N	Basic N	Humin N				
Leaves	%	%	*mg.*	%	%	%	%	*mg.*	%	%	%	%	%	*mg.*	%	*mg.*	*mg.*
Mature																	
C1 (Base)	2.8	97.2	0.502	28.9	11.2	13.3	75.5	0.668	38.3	10.9	62.7	14.7	11.7	0.571	32.8	1.239	1.741
C2	1.9	98.1	0.516	30.4	11.1	7.3	81.6	0.630	37.0	11.2	53.5	20.2	15.1	0.555	32.6	1.185	1.701
C3	4.8	95.2	0.187	11.9	13.7	11.2	75.1	0.640	40.7	10.9	35.5	29.6	24.0	0.743	47.4	1.383	1.570
C4	11.6	88.4	0.086	3.0	10.0	12.2	77.8	0.825	28.9	7.0	55.5	24.7	12.8	1.946	68.1	2.771	2.857
C5 (Tip)	11.7	88.3	0.077	2.9	11.2	17.7	71.1	0.723	27.1	8.3	58.0	22.8	10.9	1.860	70.0	2.583	2.660
Active																	
D1 (Base)	2.8	97.2	0.473	23.0	9.1	15.2	75.7	0.990	48.0	11.2	60.8	18.9	9.1	0.599	29.0	1.589	2.062
D2	2.0	98.0	0.391	26.7	9.3	10.2	81.5	0.581	39.7	12.0	52.4	20.9	14.7	0.493	33.6	1.074	1.465
D3	6.7	93.3	0.164	11.3	13.2	8.5	78.3	0.485	33.3	10.7	53.6	20.9	14.8	0.806	55.4	1.291	1.455
D4	17.2	82.8	0.070	2.9	9.2	9.7	81.1	0.774	32.8	7.9	54.7	25.0	12.4	1.514	64.3	2.288	2.358
D5 (Tip)	14.7	85.3	0.068	2.5	9.9	11.2	78.9	0.767	27.5	7.9	62.8	16.1	13.2	1.960	70.0	2.727	2.795
Young																	
E1 (Base)	5.1	94.9	0.236	11.0	10.0	12.4	77.6	0.838	39.2	7.5	70.7	14.5	7.3	1.064	49.8	1.902	2.138
E2	5.0	95.0	0.182	13.2	11.5	11.5	77.0	0.537	38.8	10.5	62.4	14.4	12.7	0.665	48.0	1.202	1.384
E3	11.4	88.6	0.079	5.0	16.2	15.4	68.4	0.377	24.2	8.4	62.1	16.8	12.7	1.102	70.8	1.479	1.558
E4 (Tip)	25.5	74.5	0.039	1.8	17.1	16.6	66.3	0.415	20.0	8.2	62.4	16.3	13.1	1.621	78.2	2.038	2.075
Stem																	
Medial																	
Cortex C	10.1	89.9	0.426	15.1	6.7	10.7	82.6	1.488	52.7	14.2	49.3	19.3	17.2	0.910	32.2	2.398	2.824
Pith C	10.5	89.5	0.492	15.8	5.0	10.3	84.7	1.841	59.0	12.1	52.8	21.6	13.5	0.786	25.2	2.627	3.119
Apical																	
Cortex D	11.9	88.1	0.302	11.0	8.6	13.3	78.1	1.498	54.4	11.0	65.4	13.2	10.4	0.954	34.6	2.452	2.754
Pith D	11.2	88.8	0.400	11.9	8.4	16.4	75.2	1.996	59.4	12.0	60.5	17.4	11.1	0.966	28.7	2.962	3.362

TABLE VIII

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT SECTIONS TOGETHER WITH PERCENTAGE VALUES OF THE SAME AND OF THEIR FRACTIONS AS FOUND IN PINEAPPLE PLANTS GROWN IN SOIL CULTURES RECEIVING NITRATE NITROGEN

Plant sections	Inorganic				Soluble organic					Insoluble organic						Total organic N	Total N
	Percentage of inorganic		Total	Percentage of total	Percentage of soluble organic			Total	Percentage of total	Percentage of insoluble organic				Total	Percentage of total		
	NH_4	NO_3			Glutamine	Asparagine	Rest N			Amide N	Monoamino N	Basic N	Humin N				
Leaves	%	%	mg.	%	%	%	%	mg.	%	%	%	%	%	mg.	%	mg.	mg.
Mature																	
C1 (Base)	4.0	96.0	0.731	.....	3.1	8.3	86.6	0.847	.....	.....	.....	.....	.....	.....	.....	.....	.....
C2	1.9	98.1	0.524	.....	5.5	8.1	86.4	0.850	.....	.....	.....	.....	.....	.....	.....	.....	.....
C3	9.8	90.2	0.102	.....	6.5	11.0	82.5	0.742	.....	.....	.....	.....	.....	.....	.....	.....	.....
C4	22.8	77.2	0.044	.....	7.4	11.6	81.0	0.645	.....	.....	.....	.....	.....	.....	.....	.....	.....
C5 (Tip)	32.0	68.0	0.050	.....	7.3	14.5	78.2	0.565	.....	.....	.....	.....	.....	.....	.....	.....	.....
Active																	
D1 (Base)	5.4	94.6	0.501	24.5	3.9	11.8	84.3	0.977	47.8	15.6	56.0	19.0	9.4	0.626	27.7	1.544	2.045
D2	4.3	95.7	0.418	25.0	8.3	9.7	82.0	0.601	36.0	13.8	47.5	30.6	8.1	0.653	39.0	1.254	1.672
D3	49.0	51.0	0.063	3.6	9.6	13.4	77.0	0.523	29.9	13.6	35.6	35.8	15.0	1.162	66.5	1.685	1.748
D4	45.5	54.5	0.066	3.0	9.3	16.0	74.7	0.344	15.8	9.1	53.0	26.1	11.8	1.759	81.2	2.103	2.169
D5 (Tip)	47.5	52.5	0.074	3.3	10.5	18.0	71.5	0.362	16.2	7.2	51.8	27.8	13.2	1.798	80.5	2.160	2.234
Young																	
E1 (Base)	17.9	82.1	0.264	13.0	4.4	11.2	84.4	0.910	44.6	11.6	61.4	19.2	8.8	0.863	42.4	1.773	2.037
E2	13.0	87.0	0.177	12.6	7.1	12.0	80.9	0.451	31.9	9.3	62.4	16.9	11.4	0.785	55.5	1.236	1.413
E3	18.9	81.1	0.053	3.0	13.7	22.1	64.2	0.366	20.6	8.5	57.8	21.6	12.1	1.348	76.4	1.714	1.767
E4 (Tip)	34.7	65.3	0.049	2.3	18.1	21.9	60.0	0.392	19.0	9.1	54.6	27.0	9.3	1.629	78.7	2.021	2.070
Stem																	
Medial																	
Cortex C	3.5	96.5	1.014	27.3	3.3	11.1	85.6	1.670	44.8	9.7	57.0	18.9	14.4	1.039	27.9	2.709	3.723
Pith C	3.2	96.8	1.099	27.1	2.7	11.2	86.1	2.001	49.2	9.9	58.3	20.6	11.2	0.960	23.7	2.961	4.060
Apical																	
Cortex D	9.7	90.3	0.718	21.7	6.1	20.0	73.9	1.454	44.1	10.2	59.0	21.8	9.0	1.131	34.2	2.585	3.303
Pith D	3.2	96.8	0.986	26.0	5.0	21.4	73.6	1.753	46.4	11.9	61.2	17.2	9.7	1.046	27.6	2.799	3.785

evidence of the oxidation of ammonium to nitrate. Both lots of plants contained great amounts of nitrate and soluble amino and basic nitrogen which, on the basis of our previous studies (**6**), indicate that both ammonium and nitrate ions were possibly absorbed simultaneously from the soil. In the absence of analytical data on plants receiving both ammonium and nitrate ions in solution cultures, we cannot explain other differences between the two lots of plants. Tables VII and VIII, presenting a more comprehensive analysis of the data in table V, show the following conditions:

1. That the percentage of total nitrogen of the stem was considerably greater in the nitrate than in the ammonium series. The percentage of nitrogen of the leaves was either of the same magnitude or slightly greater in the ammonium than in the nitrate series. The percentage of total nitrogen of the plants in the ammonium series was less when grown in soil than in solution cultures (**6**). It is possible that this condition might have been the result either of the oxidation of ammonium to nitrate which decreased the rate of absorption, on the basis of previous data (**6**), or possibly of the greater weight of the ammonium lot of plants, as compared with that of the nitrate lot, attained in soil cultures.

2. The percentage values of protein nitrogen as shown in figures 10 and 11 are higher for the plants receiving nitrate than for the plants receiving ammonium nitrogen. For the soluble organic nitrogen content, however, the values are reversed, except in the non-chlorophyllous basal sections. Inorganic nitrogen percentage values are slightly greater in the ammonium than in the nitrate series with the exception of the non-chlorophyllous basal section.

3. The sugar values in the different sections of the two lots of plants present no outstanding differences but the relatively high percentage of dry matter in the plants supplied with nitrate necessarily means greater carbohydrate synthesis although, excepting sugars, no other carbohydrates were determined.

In view of the above consideration of the experimental data, no outstanding differences can be assigned to the chemical composition of the tissues of plants grown in non-sterilized soil cultures and receiving either ammonium or nitrate salts as sources of nitrogen. The lack of such differences was possibly due to the oxidation in the soil of ammonium to nitrate.

Comparing the results of these studies with those obtained previously, one may safely state that a very great portion of the ammonium of salts which had been added to the soils, was rapidly converted to nitrate and then was absorbed as such by the plants. That some ammonium was apparently absorbed in its native state is suggested by the greater percentage values for the combined fractions of amino and basic nitrogen in the chlorophyllous sections of leaves of the groups C and D of the ammonium series than for

the nitrate series of plants. This could well be the result of ammonium absorption (**6**).

Summary

Pineapple plants grown in non-sterilized soil cultures and receiving either ammonium or nitrate salts as sources of nitrogen weighed more when grown in the cultures receiving ammonium than in those receiving nitrate.

The results of the analyses of leaf and stem sections indicated that the chemical composition of the different sections of the leaves and stem of the plants of both series, with minor exceptions, was about the same.

The most outstanding feature of these studies was the abundance of nitrate in the stem and in the non-chlorophyllous basal leaf tissues of the leaves of plants supplied with ammonium. Similar plants grown previously in nitrate-free solution cultures with ammonium salts contained no nitrate in their tissues but great amounts of amino nitrogen. The nitrate found in the tissues of the plants supplied with ammonium in the present studies was derived from ammonium which had been oxidized in the soil by microorganisms. When nitrate is absorbed by the roots from solution cultures it is transported in its native state through the tissues of the roots and stem to the chlorophyllous parts of the leaves wherein it is readily assimilated. The oxidation of ammonium salts added to soils is indicated by the presence of abundant nitrate in the non-chlorophyllous tissues of the plants.

The protein content of the leaves of plants of the nitrate series was slightly greater than that of those of the ammonium group. The soluble organic nitrogen was found to be higher, however, in the plants of the ammonium than in those of the nitrate series.

As ammonium is assimilated very quickly, inorganic nitrogen consisted mostly of nitrate; it was high in the stem and in the non-chlorophyllous sections of the leaves of the plants of both series of soil cultures.

Contrasting these findings with those of preceding studies it is safe to state that the chemical composition of plants grown in non-sterilized soil and receiving either ammonium or nitrate salts as sources of nitrogen varies very little, because of the conversion of ammonium to nitrate by the nitrifying bacteria of the soil.

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EFFECT OF TEMPERATURE UPON THE RATE OF ELONGATION OF THE STEMS OF ASPARAGUS GROWN UNDER FIELD CONDITIONS

C. W. CULPEPPER AND H. H. MOON

(WITH FIVE FIGURES)

Introduction

In connection with investigations on the use of asparagus (*Asparagus officinalis* Linn.) as a food product (**2**), an opportunity was afforded to study some of the factors that influence or control its growth. All tests were confined to field-grown material. Since the effect of temperature upon the rate of growth of asparagus in the field is of considerable practical as well as physiological interest, it was decided to make a sufficient number of measurements to permit accurate determination of the relationship of the rate of elongation of the stem or stalk to the prevailing temperatures. In general, the method has been to study the behavior of a large number rather than that of individual plants. The procedure employed was to record the climatic variations that occurred and the behavior of the plants under these conditions, and then to correlate the values obtained in the two sets of records, a method frequently employed by investigators.

Studies have been made upon the rate of growth of the stem of asparagus by BÜCHNER (**1**), TIEDJENS (**9**), WICHERS and TOLLENS (**11**), and WORKING (**12**) but nó very thorough study has been made of the effect of temperature upon the behavior of field material in mass.

Materials and methods

The material employed in these studies was grown at the Arlington Experiment Farm, Arlington, Virginia, during the seasons 1928, 1932, and 1933. The plot, approximately $\frac{1}{10}$ of an acre, had been planted in 1919, so that when the first tests were made, the planting was nine years old. The soil was a deep sandy loam of good fertility formed by dredging operations along the Potomac River. The plants were vigorous and healthy, and generally continued growth until they were 230 to 250 cm. in height. The variety was a strain of Martha Washington used in some of the breeding and selection work conducted in the U. S. Department of Agriculture.

The air temperature of the field was recorded in °F. by means of a carefully calibrated thermograph placed in a Weather Bureau instrument shelter in the center of the plot. Records were kept for the entire cutting season from about April 1 to June 15, and as usual, fluctuations in the temperature were rather wide and irregular. The lowest temperature recorded was approximately 45° F. and the highest 94° F. The temperatures given in the

tables and charts for any growth period are the hourly means for the respective periods.

Methods of measuring growth

The rate of growth was determined by direct measurement of stalk height, using a rule graduated in mm. A small stake was driven into the soil beside the stalk so that its upper end was about 2.5 cm. below the surface of the soil, and all measurements of growth of the stalk were made from the top of this stake as a base. The measurements were begun at the time, or very soon after, the stalks appeared above ground and in most cases were continued until growth had practically ceased. The measurements were usually made once each day at approximately 9:30 A.M. In a number of cases an additional reading was made at about 4:00 P.M. All stalks of the plot not being measured were cut at regular intervals as in commercial practice. Cutting was discontinued May 17, while in commercial practice it is usually continued until about June 15.

The measurements were begun on April 20, or shortly after the first stalks began to appear above the ground, and continued until the latter part of June.

The total elongation of the stalks for any period was determined by taking the difference in height at the beginning and at the end of the time interval. The height for the growth interval was calculated as the average of the height at the beginning and at the end of the interval.

To determine the rate of elongation in different regions, marks were made with a small brush and waterproof ink at suitable intervals on the bases of the leaf scales, generally from 0.5 to 1 cm. apart, in the rapidly growing part of the stalk. The increase in the length of the segments thus marked was determined for successive time intervals. The elongating part of the stalk was divided into 5 to 15 segments, the number in each case depending upon the length of the growing region. As the stalks increased in height new marks were made from time to time near the tip to keep the intervals of measurement appropriately located along the growing zone. The method was essentially that originally employed by Sachs (8) in his studies of the grand period of growth of plants.

At the beginning of the season measurements were started on about a dozen stalks which were identified by means of numbered tags. Every second or third day thereafter 2 to 5 new stalks just showing above the ground were tagged and measurements begun. Soon stalks of all heights were being measured at every period. In all, several thousand measurements were made upon a total of 130 stalks.

Methods of analyzing and plotting data on growth rates

Total elongation of the stalk.—It was obvious from inspection of the data that individual stalks varied considerably in the ultimate height reached

and in their rate of elongation. In order to determine the general behavior and to get the mean values, it appeared to be advantageous to employ a system of cross classification. The data were first classified according to the prevailing temperatures of the growth periods. Five-degree class intervals were established, extending over the range from 50° to 90° F. This gave eight temperature groups.

Inspection showed that each group included data for stalks of all heights and that the rate of elongation at a given temperature was different in stalks of different heights. The measurements in each temperature group were then classified according to the height of the stalk, as follows: All values for the growth of stalks below 10 cm. in height were put into one class, those between 10 and 20 cm. were put into another class, and so on at 10 cm. intervals until stalks 80 cm. in height were reached; then, 20 cm. classes were used up to 200 cm. in height. The values in each class were then averaged and tabulated in a manner similar to that of table I. There were 14 classes for each of the eight temperature groups, making 112 classes in all. The number of measurements in each of the classes ranged from 10 to 64. The smallest number of values in any class was for the taller stalks at the lower temperatures.

These values were plotted in two ways: the rate of growth against the tem-

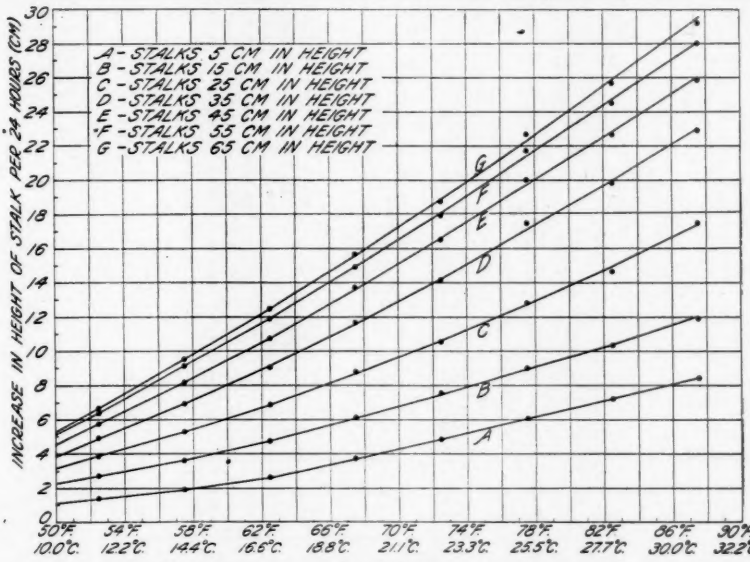

FIG. 1. Effect of temperature on the total elongation of asparagus stalks of various heights between 5 and 70 cm. The plants were grown under field conditions and the results are expressed as cm. growth per 24 hours. Average height of stalks for the 24-hour period.

TABLE I

RATE OF GROWTH OF ASPARAGUS STALKS AT DIFFERENT HEIGHTS AND TEMPERATURES, EXPRESSED IN CM. OF ELONGATION PER 24 HOURS

AVERAGE TEMPERATURE	AVERAGE HEIGHT OF STALK (CM.)													
	5	15	25	35	45	55	65	75	90	110	130	150	170	190
	RATE OF GROWTH													
°F.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
52.5	1.35	2.6	3.9	4.9	5.8	6.4	6.6	6.3	5.5	4.5	3.5	2.65	2.1	1.55
57.5	1.90	3.6	5.3	6.9	8.2	9.1	9.5	9.0	7.85	6.45	5.05	3.85	3.0	2.25
62.5	2.60	4.7	6.9	9.1	10.75	11.9	12.4	12.5	10.75	8.8	6.95	5.25	4.0	2.95
67.5	3.7	6.1	8.7	11.65	13.6	14.9	15.6	15.25	13.65	11.35	8.9	6.7	5.2	3.80
72.5	4.8	7.4	10.6	14.2	16.5	18.0	18.8	18.6	16.85	13.9	10.85	8.15	6.3	4.7
77.5	6.1	9.0	12.7	17.3	19.85	21.5	22.45	22.15	20.3	16.8	13.0	9.7	7.55	5.65
82.5	7.2	10.4	14.9	20.05	22.8	24.65	25.8	25.5	23.45	19.5	15.15	11.3	8.9	6.65
87.5	8.4	12.0	17.45	22.95	25.95	28.0	29.35	29.0	26.9	22.45	17.4	13.1	10.4	7.85

perature, giving a series of 14 curves; and against the height of the stalk, giving a series of eight curves. The first method of plotting is shown in figures 1 and 2, and the second in figure 3.

When the growth rates were plotted against either temperature or height, the points did not fall exactly upon a smooth curve. By means of a spline, or curve rule, a smooth curve was drawn through the points in such a manner that the sum of the squares of the deviations was as nearly a minimum as could be estimated by simple arithmetical calculations.

The values were plotted upon coordinate paper upon a large scale. The new average or adjusted values of the rates of growth for the midpoints of each of these 112 classes were read from the chart for both systems of curves. Obviously, these values should be the same in both systems of curves but they were not identical in many cases, some being higher and some lower in one system than in the other. The two values for each of the corresponding points were averaged and the new values tabulated in the same way as was done at first. These new values were again plotted as had been done in the first place and new curves drawn in the same way. When the values were read from the new curves they agreed much more closely than at first. After plotting and averaging in this way three times, the values (table I) finally obtained, when plotted, form loci of points that lie almost exactly upon smooth curves. They represent estimated mean values of the growth rates of the stalks of asparagus in this plot as a whole.

DIFFERENT REGIONS ALONG THE STALK.—In order to avoid the presentation of an excessive amount of tabular matter, the data for rate of growth in different regions of the stalk were classified in the same manner as the data for the total elongation of the stalk. A particular stalk in each of these classes had, instead of one reading as in the values for the total elongation, a series of readings made at various points from base to tip. The values for the growth rates at corresponding points along the stalks of all the individuals in the class were averaged. This was done at a sufficient number of points (7 to 12) to permit plotting of the results. When these values were plotted, the line represented the rate of growth along the entire growing region for the stalks of a particular height at a particular temperature. Obviously, to plot the rates of growth for 112 classes would require a great amount of space. Consequently, the data for the growth rates of stalks of only two heights, 15 and 75 cm., have been calculated for the temperatures between 52.5° and 77.5° F. This is believed to be sufficient to illustrate the general character of the results.

Observations

EFFECT OF TEMPERATURE UPON TOTAL ELONGATION

After making a few measurements it was obvious that there was a very definite positive correlation between the growth of asparagus and the prevail-

ing temperatures as shown in table I. The results for all plants 70 cm. or less in height are illustrated in figure 1, while those for plants more than 70 cm. in height are shown in figure 2. It is evident that the relationship between temperature and total elongation per 24 hours is in all cases represented by nearly straight lines. They are slight curves which slope upward a little more sharply in the portion of the curves representing the higher temperatures. This is true for stalks of all heights. The form of the curve

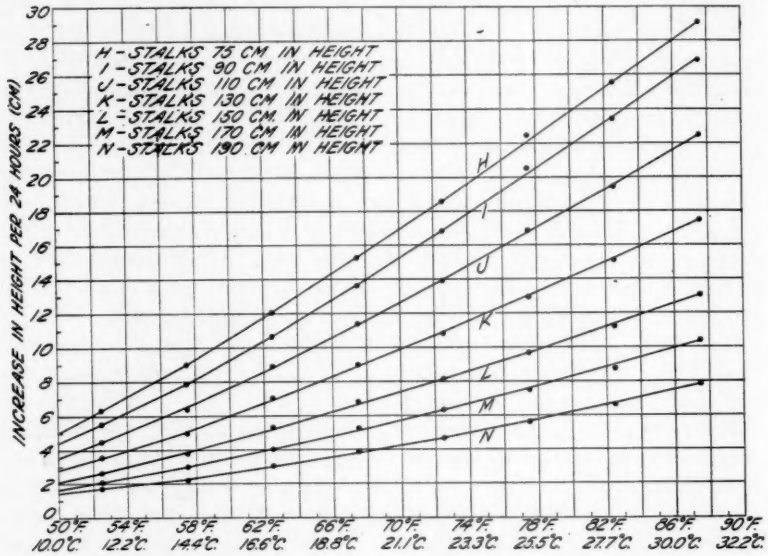

FIG. 2. Effect of temperature on the total elongation of asparagus stalks of various heights between 70 and 200 cm. The plants were grown under field conditions and the results are expressed as cm. growth per 24 hours. Average height of stalks for the 24-hour period.

is nearly the same whatever the height of the stalk may have been at the time of measurements. The difference in slope of the lines indicates that stalks 60 to 70 cm. high are a little more sensitive to changes in temperature than those that are taller or shorter. The reason for this is not clear but it seems to be associated with the length of the growing zone. Also the very old stalks are not quite so sensitive to increases in temperature as are the very young stalks. This may be due in part to failure to transport water and food materials to the growing region of the tall stalks at the higher temperatures as rapidly as the materials are required for the formation of new tissues. It would be of interest to have comparable data on material with the prevailing temperatures both higher and lower than those which prevailed under these

TABLE II

RATE OF GROWTH AT VARIOUS 10° C. INTERVALS AND THE RATIO OF THE RATE OF GROWTH AT THE END TO THAT AT THE BEGINNING OF THE INTERVAL

TEMPERATURE	HEIGHT OF STALKS IN CM.														
	5			25			65			130			190		
	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a
	LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b	
°C.	*cm.*	*cm.*		*cm.*	*cm.*		*cm.*	*cm.*		*cm.*	*cm.*		*cm.*	*cm.*	
10 and 20	1.08	3.77	3.49	3.18	8.81	2.77	5.28	15.90	3.01	2.80	9.03	3.22	1.43	3.90	2.72
11 and 21	1.30	4.18	3.21	3.65	9.50	2.60	6.27	17.04	2.88	3.30	9.80	2.97	1.60	4.20	2.62
12 and 22	1.50	4.60	3.06	4.20	10.20	2.43	7.29	18.24	2.50	3.81	10.52	2.76	1.80	4.50	2.50
13 and 23	1.68	5.02	2.98	4.70	10.95	2.33	8.30	19.43	2.34	4.40	11.28	2.56	1.96	4.86	2.47
14 and 24	1.87	5.48	2.92	5.20	11.70	2.25	9.33	20.63	2.21	4.98	12.02	2.41	2.20	5.18	2.35
15 and 25	2.10	5.90	2.81	5.72	12.43	2.17	10.38	21.88	2.10	5.60	12.80	2.28	2.47	5.54	2.24
16 and 26	2.36	6.33	2.68	6.30	13.22	2.10	11.41	23.17	2.03	6.27	13.59	2.16	2.72	5.90	2.20
17 and 27	2.64	6.75	2.56	6.91	14.03	2.03	12.52	24.43	1.95	6.94	14.38	2.07	3.00	6.30	2.10
18 and 28	2.97	7.20	2.42	7.43	14.92	2.01	13.50	25.80	1.91	7.54	15.22	2.01	3.26	6.7	2.07
19 and 29	3.38	7.60	2.26	8.15	15.75	1.93	14.72	27.06	1.84	8.32	15.96	1.92	3.58	7.08	1.98
20 and 30	3.77	8.02	2.12	8.80	16.63	1.89	15.88	28.37	1.79	9.04	16.74	1.84	3.90	7.48	1.92
21 and 31	4.18	8.50	2.03	9.51	17.6	1.84	17.04	29.60	1.73	9.80	17.55	1.79	4.20	7.90	1.88

conditions. By extrapolation of the curves it may be inferred that below 40° F. very little or no growth will occur. It is also evident that at 87.5° F. (30.8° C.), the highest average temperature recorded, the rate of elongation had not reached its maximum. From the slope of the curves it appears unlikely that under field conditions, temperatures high enough for maximum rate of elongation will often prevail in this latitude.

In order to see how closely the rate of elongation of the growing asparagus stem conforms to the VAN'T HOFF-ARRHENIUS principle (**10**) the ratio of the rate of elongation at the end of various 10° C. (18° F.) intervals of temperature to that at the beginning has been calculated. According to this principle, the rate of growth may be expected to double for each 10 degrees of increase. It may be noted in table II that, over a considerable range of temperature, the rate of elongation of the stem does roughly double for an increase of 10° C. The rate almost exactly doubles as the temperature is raised from 16° to 26° C. for stalks of all heights except those 5 cm. tall. In the upper range of the temperatures prevailing in this study, the rate of elongation fails to double, while for intervals in the lower range it more than doubles for each rise of 10° C. It is apparent that this relationship between the prevailing temperature and the growth rate does not conform exactly to

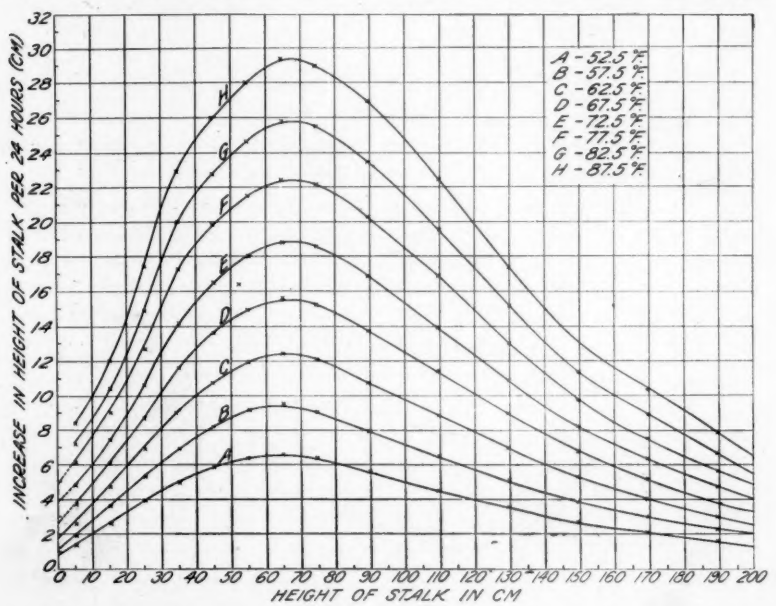

FIG. 3. Rate of growth of asparagus stalks at different heights for various temperatures expressed in cm. per 24 hours.

the VAN'T HOFF-ARRHENIUS principle. It may be inferred however, that it would be advantageous for a grower to cut the field twice as often at 26° C. (78.8° F.) as at 16° C. (60.8° F.).

RELATION OF HEIGHT OF STALK TO RATE OF GROWTH

Many writers have noted that the rate of growth varies continuously with differences in age or size of the plant. In the case of asparagus, WORKING (**12**) has presented the relationship very clearly. It may be noted from figures 1 and 2 that the stalks intermediate in height elongate much faster than the very tall or the very short ones. The relationship of the height of the plant to the rate of elongation is shown much more clearly in figure 3. As the material increases in height, the rate of elongation increases rapidly to a maximum and then gradually decreases to low values in the very tall stalks. The height of the stalk at which elongation is at a maximum is between 60 and 70 cm. for any given temperature.

Under the conditions of these measurements, the temperature might be low one day and high the next, or might vary in almost any manner. The present results are consequently a record of the response of plants of different heights to changes of temperature, and it should be remembered that the same magnitudes of change cannot be expected with material grown under uniform conditions of temperature throughout the entire period of their growth.

SUMMATION OF THE TEMPERATURE

It is clear from the above discussion that the height of the stalk at any time is a function of both time and temperature. It has often been considered that the development of the plant is dependent upon the amount of heat that it receives, the amount of heat being dependent upon the temperature and the length of the exposure. This idea has led to the working out of several systems of temperature indices (**5, 7**). One of the first of these is known as the remainder system, in which effective heat is estimated as total degree-hours above an arbitrary base temperature. The most essential thing in the employment of this system is the establishment of a correct temperature at which to begin counting the heat units. It has often been considered that the proper temperature for use as a base line is that at which growth first occurs. This may not always be the most practical or workable base line, as has been suggested by MAGOON and CULPEPPER (**6**). By extrapolation of the curves in figures 1 and 2 it may be inferred that growth in asparagus first becomes appreciable somewhere near 40° F., but no attempt was made to determine definitely the most appropriate base line. After considering the data to some extent it was concluded that 42.5° F. was fairly suitable, and using this as a base line, the temperatures have been summated for stalks 20, 50, 100 and 150 cm. in height, at 8 temperatures differing by 5° F. The results are shown in table III.

TABLE III

DEGREE-HOURS REQUIRED FOR ASPARAGUS STALKS TO GROW TO VARIOUS HEIGHTS WHEN EXPOSED TO VARIOUS ATMOSPHERIC TEMPERATURES, BEGINNING WITH STALKS 2.5 CM. BELOW THE SURFACE OF THE SOIL

HEIGHT OF STALK	GROWTH TEMPERATURE IN °F.							
	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5
	DEGREE-HOURS ABOVE 42.5° C.							
cm.								
20	3000	3312	3226	3000	2736	2688	2688	2700
50	4608	5076	4848	4860	4680	4512	4608	4536
100	6564	7164	7128	6900	6660	6510	6480	6480
150	9792	10476	10320	9960	9648	8324	9180	9660

It may be noted that roughly the same number of degree-hours are required for a stalk to grow from the base (2.5 cm. below the surface of the soil) to any given height for any temperature between 52.5° and 87.5° F. A perfect agreement could not be expected as the growth temperature relationship is only approximately linear. According to these calculations about 2900 degree-hours are required for a stalk to grow from the base to a height of 20 cm. and about 9800 degree-hours to grow to 150 cm. in height.

TIME REQUIRED FOR STALKS TO GROW TO DIFFERENT HEIGHTS

Knowing the rate of elongation of the stalk at all heights at every temperature, it is a simple matter to calculate the time required to reach any given height. By summating the growth rates day after day the number of days required to reach various heights have been obtained for material growing at various temperature levels. This was done by adding to the height of the plant at the end of the first day the growth made during the second day, which gives the height of the plant at the end of the second day, and so on until the ultimate height is reached. The data are given in table IV, and figure 4 shows very clearly the effect of temperature upon the time necessary to reach different heights. It may be expected that 6 days will be required for plants to reach a height of 100 cm. when the temperature averages 87.5° F.; 11.5 days when it averages 67.5° F.; and 27.2 days when it averages 52.5° F. The curves for 82.5 and 87.5° F. are of theoretical interest only for it is improbable that an average temperature as high as 87.5° F. for any 24-hour period would occur during the harvest season of asparagus. It must also be remembered that in this case the average daily temperature was never as high as 87.5° F. or as low as 52.5° F. for the entire growth period of any stalk and therefore these values will probably not hold for long periods of growth at the indicated temperatures. It is believed they will hold for short periods of growth and therefore may be of

TABLE IV

ESTIMATED HEIGHT OF STALKS OF ASPARAGUS AFTER GROWING FOR DIFFERENT LENGTHS OF TIME AT VARIOUS TEMPERATURES. ESTIMATED FROM THE GROWTH RATES AT DIFFERENT TEMPERATURES

GROWING PERIOD	GROWTH TEMPERATURE IN °F.							
	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5
	HEIGHT OF STALK							
Days	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
0	0	0	0	0	0	0	0	0
2	1.5	2.2	3.6	5.5	8.1	10.8	13.2	15.7
4	3.4	5.3	8.9	14.0	20.8	29.0	36.7	47.2
6	5.9	9.4	16.7	27.2	42.5	63.0	82.3	102.7
8	9.1	15.3	28.0	48.5	76.9	106.3	127.2	145.0
10	13.2	23.3	45.0	78.2	111.4	138.0	155.5	171.0
12	18.5	34.0	67.6	106.4	136.6	159.4	175.4	190.2
14	25.1	48.3	91.7	128.6	155.1	175.7	191.0	204.8
16	33.3	66.1	111.7	145.8	169.7	188.9	203.3	
18	43.5	84.5	128.1	159.5	181.9	200.0		
20	55.2	100.5	141.7	170.9	192.2			
22	68.1	114.2	153.0	180.7	200.8			
24	81.0	126.0	162.7	189.2				
26	92.7	136.3	171.2	196.6				
28	103.2	145.4	178.8					
30	112.6	153.4	185.6					
32	121.1	160.6	191.8					
34	128.7	167.1	197.5					
36	135.7	173.2						
40	147.9	184.2						
44	158.3	193.6						
47	165.3	200.0						
48	167.5							
54	179.7							
60	190.0							
64	196.1							
67	200.3							

some practical importance in indicating how often the crop should be cut when the cutting of the stalks is to be made between certain definite heights.

According to these measurements, for stalks to grow from 10 cm. to 25 cm. in height, 5.3 days would be required at 52.5° F., 4.2 days at 57.5° F., 3.4 days at 62.5° F., 2.4 days at 67.5° F., 2.1 days at 72.5° F., and 1.9 days at 77.5° F. If a farmer restricted the cutting to stalks between these heights, he would have to cut the field at least as often as indicated or some of the stalks would be too tall at each cutting. In other words, it may be expected that about 1400 degree-hours of temperature would be required for stalks to elongate from 10 cm. to 25 cm. Of course the degree to which this rate of production would be realized would vary considerably with the fertility and moisture content of the soil, vigor of the plants, and possibly other factors. The plants in these tests were very vigorous as shown by the fact that the stalks finally reached a height of 230 to 250 cm.

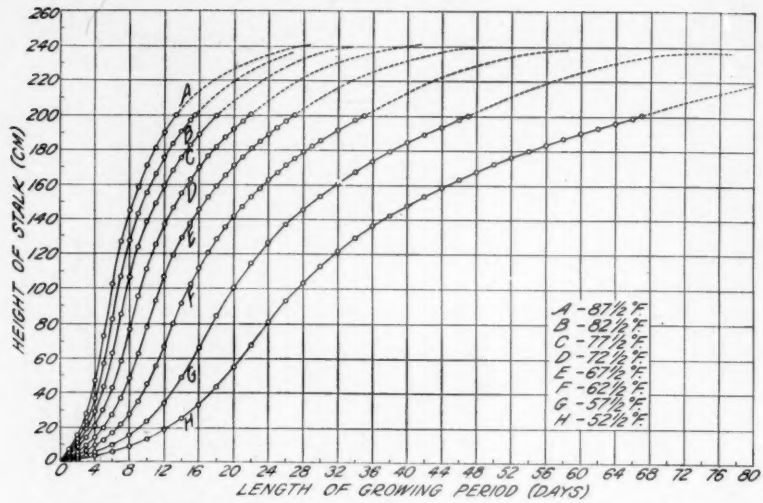

FIG. 4. Height in cm. of asparagus after growing for various lengths of time at various temperatures. The time values are expressed in days and the temperature in °F.

EFFECT OF TEMPERATURE UPON THE RATE OF ELONGATION ALONG THE STALK

The amount of elongation in different portions of the growing zone has been determined by WORKING (**12**). The rate of elongation per unit length of the stalk is maximum in a zone a short distance below the tip. The manner of growth is evident from figure 4.

It would be expected that the effect of temperature upon the rate of elongation in different zones along the stalk would be similar to the effect upon total elongation.

The results obtained from these measurements are given in table V and figure 5.

It is apparent from figure 5, L and M, that temperature affects the rate of growth of the stalk at every point throughout the entire growing region. The zone of maximum rate of growth, however, seems to be somewhat more sensitive to changes in temperature than zones above or below. The difference in sensitiveness is apparently small and may not be significant.

The curves indicate that growth stops at a point somewhat higher on the stalk at the low temperature than at the higher temperature. This may be attributed to error in selecting stalks for measurement.

The effect of the temperature upon the rate of growth at any point along the stalk appears to be approximately the same as for the total elongation, as shown in figures 1 and 2. In figure 5, N, the rates of growth at 5 different

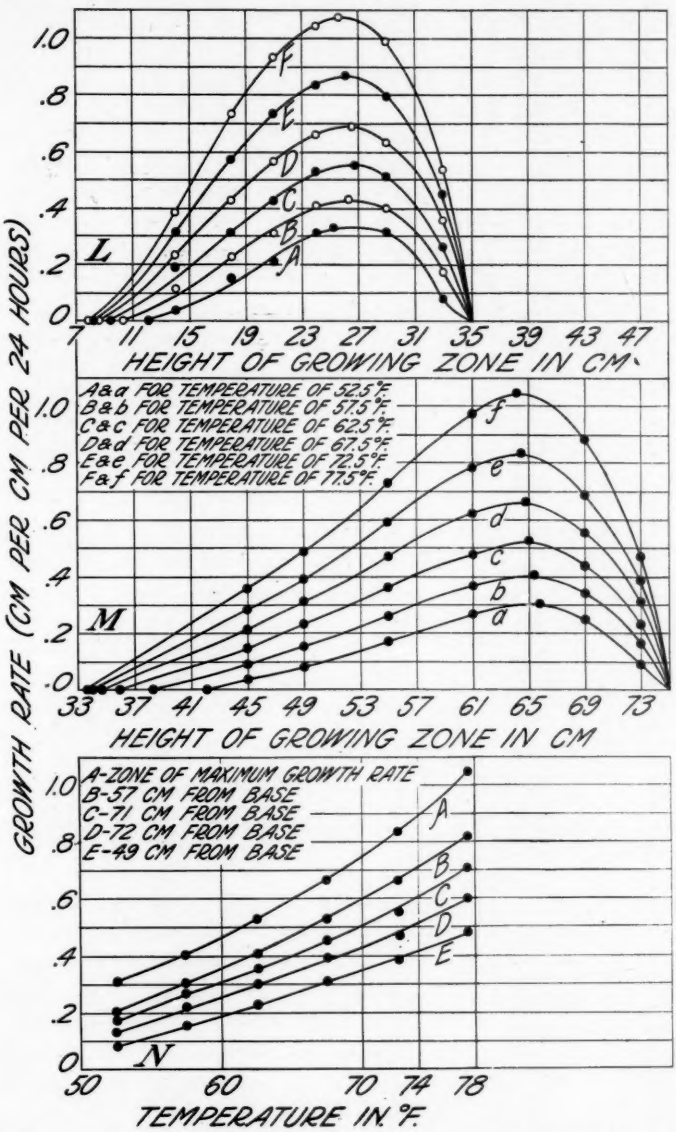
GROWTH RATE (CM PER CM PER 24 HOURS)
1.0
.8
.6
.4
.2
0
L
7 11 15 19 23 27 31 35 39 43 47
HEIGHT OF GROWING ZONE IN CM.
A&a FOR TEMPERATURE OF 52.5°F.
B&b FOR TEMPERATURE OF 57.5°F.
C&c FOR TEMPERATURE OF 62.5°F.
D&d FOR TEMPERATURE OF 67.5°F.
E&e FOR TEMPERATURE OF 72.5°F.
F&f FOR TEMPERATURE OF 77.5°F.
M
33 37 41 45 49 53 57 61 65 69 73
HEIGHT OF GROWING ZONE IN CM
A-ZONE OF MAXIMUM GROWTH RATE
B-57 CM FROM BASE
C-71 CM FROM BASE
D-72 CM FROM BASE
E-49 CM FROM BASE
N
50 60 70 74 78
TEMPERATURE IN °F.

FIG. 5. Effect of temperature upon the rate of growth in various zones along the stalks of asparagus after they have reached the total height of 35 and 75 cm. L, stalks 35 cm.; M, stalks 75 cm. in height. N, the effect of temperature upon the rate of growth in different regions of stalks 75 cm. in height.

TABLE V

RATES OF GROWTH ALONG THE STALK OF ASPARAGUS UNDER VARIOUS TEMPERATURE CONDITIONS FOR STALKS 35 AND 75 CM. TALL, EXPRESSED IN CM. PER CM. PER 24 HOURS

TOTAL HEIGHT OF STALK	TEMP.	HEIGHT OF GROWING SEGMENT	RATE OF GROWTH	HEIGHT OF GROWING SEGMENT	RATE OF GROWTH	HEIGHT OF GROWING SEGMENT	RATE OF GROWTH	HEIGHT OF GROWING SEGMENT	RATE OF GROWTH	HEIGHT OF GROWING SEGMENT	RATE OF GROWTH	HEIGHT OF GROWING SEGMENT	RATE OF GROWTH
cm.	°*F.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
35	52.5	12.1	0.000	15.0	0.055	17.0	0.101	19.0	0.160	21.0	0.224	23.0	0.283
"	57.5	10.3	0.000	15.0	0.118	17.0	0.187	19.0	0.257	21.0	0.323	23.0	0.379
"	62.5	9.3	0.000	13.0	0.112	16.0	0.230	19.0	0.352	21.0	0.435	23.0	0.498
"	67.5	8.6	0.000	13.0	0.175	16.0	0.327	19.0	0.474	21.0	0.565	23.0	0.636
"	72.5	8.1	0.000	11.0	0.115	14.0	0.315	17.0	0.510	20.0	0.680	23.0	0.804
"	77.5	7.9	0.000	11.0	0.150	14.0	0.385	17.0	0.635	20.0	0.870	23.0	1.064
"	52.5	25.0	0.325	25.2	0.328	27.0	0.325	29.0	0.310	31.0	0.233	32.0	0.167
"	57.5	25.0	0.418	26.3	0.431	27.0	0.429	29.0	0.400	31.0	0.320	32.0	0.253
"	62.5	25.0	0.537	26.8	0.551	27.0	0.550	29.0	0.510	31.0	0.415	32.0	0.343
"	67.5	25.0	0.678	26.6	0.689	27.0	0.683	29.0	0.630	31.0	0.540	32.0	0.460
"	72.5	25.0	0.852	26.2	0.864	27.0	0.858	29.0	0.790	31.0	0.663	32.0	0.563
"	77.5	25.0	1.064	25.6	1.073	27.0	1.063	29.0	0.985	31.0	0.832	32.0	0.710
75	52.5	42.2	0.000	45.0	0.037	49.0	0.080	53.0	0.138	57.0	0.202	59.0	0.237
"	57.5	38.2	0.000	45.0	0.090	49.0	0.154	53.0	0.220	57.0	0.301	59.0	0.338
"	62.5	36.0	0.000	41.0	0.078	45.0	0.145	49.0	0.230	53.0	0.318	57.0	0.405
"	67.5	35.7	0.000	41.0	0.132	45.0	0.210	49.0	0.310	53.0	0.410	57.0	0.527
"	72.5	34.0	0.000	41.0	0.174	45.0	0.280	49.0	0.390	53.0	0.522	57.0	0.667
"	77.5	33.7	0.000	41.0	0.232	45.0	0.355	49.0	0.484	53.0	0.640	57.0	0.820
"	52.5	63.0	0.285	65.8	0.301	67.0	0.284	69.0	0.245	71.0	0.177	72.0	0.134
"	57.5	63.0	0.390	65.4	0.404	67.0	0.385	69.0	0.340	71.0	0.268	72.0	0.220
"	62.5	61.0	0.477	65.1	0.524	67.0	0.494	69.0	0.438	71.0	0.356	72.0	0.300
"	67.5	61.0	0.623	64.8	0.664	67.0	0.618	69.0	0.550	71.0	0.456	72.0	0.393
"	72.5	61.0	0.780	64.5	0.839	67.0	0.778	69.0	0.687	71.0	0.550	72.0	0.475
"	77.5	61.0	0.970	64.2	1.048	67.0	0.990	69.0	0.880	71.0	0.710	72.0	0.600

locations along the stalk for material 75 cm. in height have been plotted against temperatures between 52.5° and 77.5° F. Each series of points forms loci that lie in almost a straight line. The slight curve slopes upward a little more sharply with the higher temperatures. It appears that the region of maximum growth is slightly closer to the base at high temperatures than at low temperatures. This may be a response that is related to the growth behavior early and late in the season. The differences are not large and may not be significant. These results should be interpreted as responses to variations in temperature during short intervals of time under field conditions and may not be the same as would occur under conditions where the temperature is constant. It has been shown by LEHENBAUER (3) and LEITCH (4) that the temperature for maximum growth depends upon the length of the period of exposure.

It may be concluded that the growth rate of asparagus is similar to that of many other plants and the results of these field measurements agree essentially with the results of SACHS (8) and many other investigators.

Summary

1. The effect of temperature upon the rate of elongation of asparagus stalks has been studied under field conditions. Averages of a large number of measurements have been obtained by a method of cross classification, followed by a system of two-way plotting, the final values forming smooth curves when plotted in either of the two ways.

2. For temperatures between 52.5° and 87.5° F., the relationship between the growth rate and the temperature is represented by lines that are almost straight. The rate of total elongation approximately doubled with each increase of 10° C. (18° F.) over a limited range of temperatures.

3. The relationship between the rate of total elongation and the height of the stalk has been determined for the stalks in these tests and the results plotted. The rate of increase in height was slow at first, increased rapidly to about 65 cm. in height when it was at a maximum, and then slowly decreased as the stalks became taller.

4. The growth responses of these plants have been interpreted in terms of time required to reach different total heights. The lines representing this relationship take the form of the S-curve characteristic of growth processes. It may be seen from these rates that if it is desired to cut asparagus between the heights of 10 and 25 cm. the field would need to be cut at least every 5.3 days when the average temperature was 52.5° F., every 4.2 days when the average was 57.5°, every 3.4 days when it was 62.5°, every 2.4 days when it was 67.5°, and every 2.1 days when it was at 72.5° F. Likewise the time required for growth from any initial height to any other height at any designated temperature may be readily ascertained from the curves.

5. Variations in temperature affect the rate of growth along the stalk in about the same way as they affect the total elongation. The growth rate increases for a short distance below the tip to a point where it is maximum and then decreases to the lower limits of the growing region. The results indicate that the zone of maximum growth is a little more sensitive to changes of temperature than regions either above or below this zone.

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U. S. DEPARTMENT OF AGRICULTURE

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PROBLEMS IN THE STORAGE OF CUT CARNATIONS

M. S. NEFF

(WITH TEN FIGURES)

Cut flowers are commonly stored with the stems in water. Such treatment results in a high turgor pressure within the plants which may be augmented by high humidity in the storage room. Preliminary experiments (3) indicated that the life of flowers is shortened by a liberal water supply during storage.

Materials and methods

Comparisons were made between flowers stored in water and flowers stored "dry pack." When removed to ordinary temperature and humidity conditions, keeping qualities were observed and compared with fresh flowers. The flowers were arbitrarily classed as "desirable" until the edges of the petals first began to dry, or any other noticeable defect appeared. "Percentage desirable" as used in the following graphs indicates the number of "desirable" blooms expressed in percentage. The average number of flowers used in each lot was about nine.

"Dry pack" indicates storage without water. Flowers in "dry pack" were not placed in water until removed from storage. The methods of dry packing varied considerably. Wrappings of different kinds of paper were used in an attempt to find a suitable storage condition. A more successful method consisted of storing the flowers in sealed glass or metal containers. A refinement of this procedure made possible the control of the atmosphere under bell jars. Two-hole rubber stoppers supplied with glass tubing were fitted into the openings at the tops of the bell jars. Rubber tubing fitted to the outlets and closed by means of clamps facilitated gas analysis with the HALDANE gas analyzer. The outlets were also used for aeration and the introduction of carbon dioxide.

Results

RELATION OF TURGOR TO THE KEEPING OF CUT CARNATIONS

The following experiments bring out marked differences in the behavior of carnations stored dry and those stored with the stems in water. Fluctuations in the weights of flowers were observed and the water loss per flower recorded during the period of observation at room temperature to determine the lasting qualities.

Carnations were placed in water at room temperature and humidity to determine their "desirable" length of life (fresh). Similar carnations were stored for 32 days with their stems in water. The stems were clipped

under water and the water changed 18 times during this period. (Water, 32 days.)

Carnations were also sealed in empty desiccating jars, one inverted over the other. The flowers were at no time placed in water until two hours before removal to room temperature. (Dry III, 32 days; Dry IV, 46 days.)

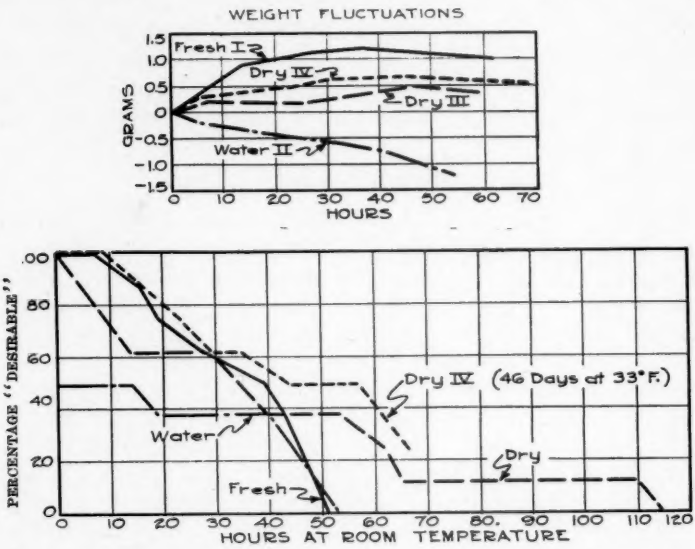

FIG. 1. "Wet" and "dry" storage carnations compared with fresh flowers at room temperature. Storage period, 32 days and 46 days at 33° F. Above, weight changes with time. Below, deterioration with time.

Carnations stored dry in sealed containers were in much better condition and lasted longer at room temperatures than carnations stored in water. On the average they stood up as well under room conditions as the freshly cut flowers.

Fresh carnations placed in water under room conditions gained in weight, reaching a maximum at the thirty-sixth hour. Carnations stored 46 days "dry pack," when removed and placed in water, underwent similar gains reaching a maximum at the forty-sixth hour. In contrast carnations stored for 32 days in water steadily lost weight at room temperature. The decrease in weight was strongly correlated with lack of keeping quality.

Flowers stored with the stems in water gained in weight and immediately had moisture available in unlimited quantities for any chemical or physical reactions. Although low temperatures greatly inhibit most plant processes, carnations stored in water tend to complete their developmental cycle. On

the other hand, "dry pack" flowers with judiciously lowered turgidity should have exceptional keeping qualities if moisture were a factor in hastening maturity.

Possibly more emphasis and importance should be attributed to the high carbon dioxide and low oxygen content within the sealed containers. Moderately high carbon dioxide has been found beneficial in flower storage (**6**).

It is interesting to note that the water loss at room temperature was approximately the same for fresh carnations, "dry pack" carnations, and those stored in water. This result might preclude the idea that increased life of the flowers at room temperature depended on higher transpiration.

The following determinations resemble the previous studies except no weighings were made. Fresh carnations were placed in water at room temperatures to determine the length of life. The stems of carnations stored in water were cut and the water changed several times during the storage period. The "dry pack" carnations were sealed in a dry glass jar and were at no time in water until two hours before removal to room temperature.

When carnations were placed at room temperature and humidity, the stems were cut and the water changed daily.

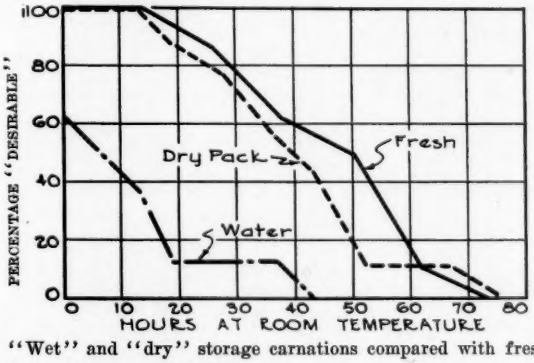

FIG. 2. "Wet" and "dry" storage carnations compared with fresh carnations at room temperature. Storage period, 37 days.

These results confirm the belief that turgor is an important factor in storage of carnations. "Dry pack" carnations compare favorably with fresh flowers as to keeping qualities at room temperature. Carnations stored with their stems in water were greatly inferior to fresh carnations.

In the following experiment additional differences in turgidity were produced by cutting the flowers in the heat of the day and in the evening, in addition to spreading the flowers out in the 40° F. room and wilting them for varying lengths of time in the relatively dry atmosphere of the room.

Fresh carnations were placed in water and "hardened" for 15 hours at

40° F. They were then placed at room temperature and humidity to determine the length of commercial life. Additional carnations were stored with the stems in water. Similar groups were precooled three and seven hours at 40° F. and sealed in dry glass containers. Because of the drying effect of the precooling room, the carnations "dry" and "dry delayed" were slightly wilted at the time of storage. The "dry pack" flowers were at no time placed in water until eight hours before removal to room temperature, when the stems were clipped, and the water was then changed every day.

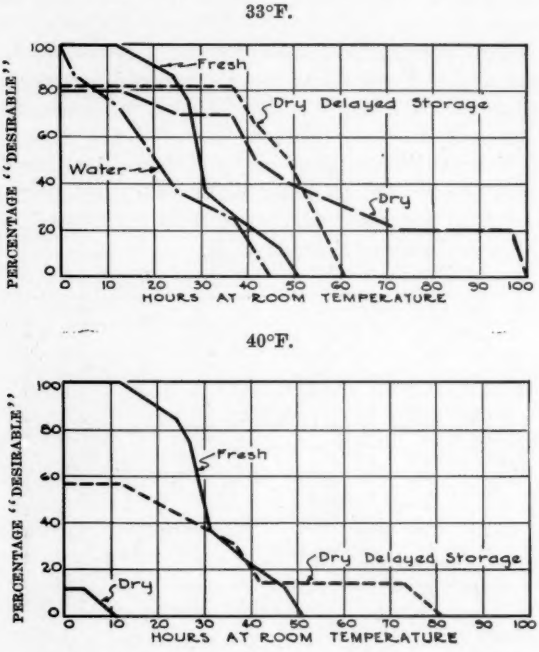

FIG. 3. "Wet" and "dry" storage carnations compared with fresh flowers at room temperature. Stored 39 days. Above, storage temperature 33° F. Below, storage temperature 40° F. "Dry delayed" were cut at noon and precooled for 7.5 hours. "Dry" were cut at 6 P. M. and precooled 3 hours. (Water stored flowers have been omitted from the 40° F. graph owing to their unsatisfactory condition.)

Carnations cut at midday and stored at 40° F. wilted slightly but were many times better than those picked in the evening, and were more turgid. It is probable that lowered turgidity dominated internal conditions and made possible the survival of the flowers. At 33° F. the differences were not so pronounced.

"Dry pack" carnations stored for 39 days lasted longer under room conditions than freshly picked flowers. This reaction is important, for the value of long-time storage depends on the life of the cut flowers after re-

moval from storage. As in previous experiments, the carnations stored dry lasted longer at room temperature than those stored in water.

The results also show the advisability of using low storage temperatures. At medium and high temperatures the respiration rate appeared to be too intense for long time storage of flowers. As the length of storage depends on minimizing respiration and other natural processes, the only alternative is the use of low temperatures.

The following graphs give additional evidence concerning water relations. Joan Marie carnations were shipped approximately 1100 miles and then placed in the low temperature room (33° F.), thus introducing a delay of 44 hours between cutting and the beginning of storage. Twelve hours later one group was sealed in a bell jar and a second lot was stored with the stems in water. The flowers in general were quite "tight," several being only one-half open at the time of storage.

Carbon dioxide was introduced into the storage bell jar to the extent of 6.4 per cent. The outlets were allowed to remain open and subsequent determinations showed that the gas percentage remained practically constant.

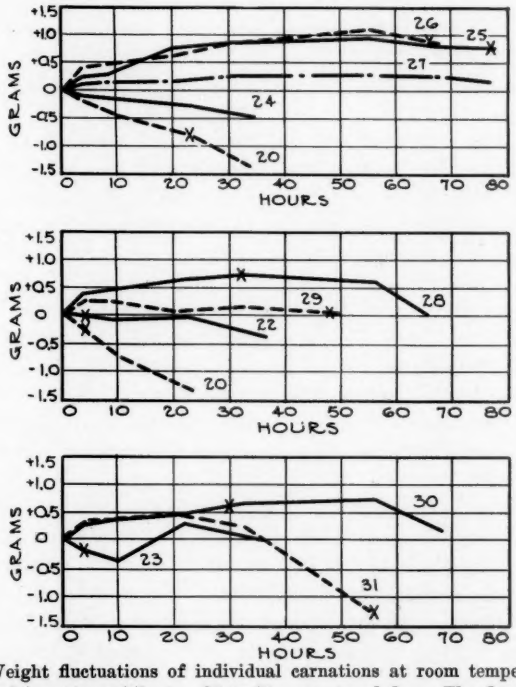

FIG. 4. Weight fluctuations of individual carnations at room temperature Nos. 20 to 24 were stored in water, while nos. 25 to 31 were stored dry. The flowers were stored 55 days at 33° F., following 44 hours delayed storage. Nos. 24 and 27 were not "desirable" at the time of removal. The position of X on each curve marks the close of the period of "desirable" condition.

Carnations stored "dry pack" were again superior to those stored in water. Previous results that the flowers showed increasing weight when placed in water were confirmed, while flowers stored in water lost weight when removed to room temperature.

There was a marked difference in the water loss at room temperature of flowers stored dry and those stored in water. It is probable that bacteria lodged in the conducting tissues in sufficient numbers to prevent the free absorption of water by the flowers stored in water (5). The water was changed only once during the 55 days of storage and as a result the stems were slimy and the water cloudy. It may be that after 55 days a combination of bacterial clogging and general breakdown occurred. A previous experiment, run for 32 days with only a few changes of water, resulted in a subsequent normal rate of water loss.

A preliminary carbohydrate analysis of the carnations shipped furnished additional information concerning turgidity. The analysis procedure was that described by LOOMIS and SHULL (2). All calculations were made on the extracted dry weight basis.

Sugars were determined from an 80 per cent. alcohol extraction (11 times). Dextrin and starch were determined from a hot water extract of the residue remaining from the alcohol extraction. The acid hydrolyzable fraction was obtained from the residue remaining from the dextrin-starch determinations.

The carbohydrates analyzed for were concentrated in the heads of the flowers. Considering heads alone, carnations stored 55 days "dry pack" were only slightly lower in total sugars than fresh carnations (table I). The same thing held true for glucose, fructose, sucrose, and reducing sugars. On the other hand, flowers stored with the stems in water were reduced in sugars by approximately one-half.

The dextrin and starch fractions of fresh and "dry pack" carnations were practically the same, while the same fractions in flowers stored in water were still considerably less. The more stable and less available acid-hydrolyzable fractions were practically the same in every case.

The analyses suggest a considerable utilization of available foods in the case of flowers stored in water, and a slight utilization when flowers were stored "dry pack."

Another group of carnations, shipped as before, were placed in the College low temperature room within 43 hours. Twelve hours later one group was sealed in a bell jar and a second lot placed in water at room temperature to determine the life of the fresh flowers. The flowers of the Joan Marie variety were tight when received; Pink Spectrum were fully open. The stored flowers were at no time in water until placed at room temperature. Carbon dioxide was held below 14 per cent. by frequent aerations.

TABLE I

PRELIMINARY CARBOHYDRATE ANALYSIS OF JOAN MARIE CARNATIONS

FRACTIONS	No. I		No. II		No. III	
	FRESH CARNATIONS		STORED 55 DAYS STEMS IN WATER		STORED 55 DAYS "DRY PACK"	
	HEADS	STEMS	HEADS	STEMS	HEADS	STEMS
Green weight	37.85 gm.	26.60 gm.	52.62 gm.	25.6 gm.	45.8 gm.	21 gm.
Extracted dry weight	4.687 "	3.952 "	4.708 "	3.476 "	4.581 "	3.2527 "
Calculated as:						
Reducing sugars	32.49%	1.97%	16.52%	0.00%	29.54%	0.00%
Sucrose	5.57 "	1.88 "	3.35 "	2.46 "	5.65 "	1.21 "
Fructose	7.08 "	0.00 "	3.56 "	0.00 "	6.48 "	0.00 "
Glucose	25.41 "	1.97 "	12.96 "	0.00 "	23.06 "	2.32 "
Total sugars	38.06 "	3.85 "	19.83 "	2.46 "	35.19 "	1.21 "
Dextrin and starch	4.90 "	2.11 "	3.84 "	1.98 "	4.86 "	2.25 "
Acid hydrolyzable	14.28 "	8.50 "	13.14 "	8.35 "	13.87 "	8.52 "
Total carbohydrates	57.24 "	14.46 "	35.84 "	12.79 "	53.92 "	11.98 "

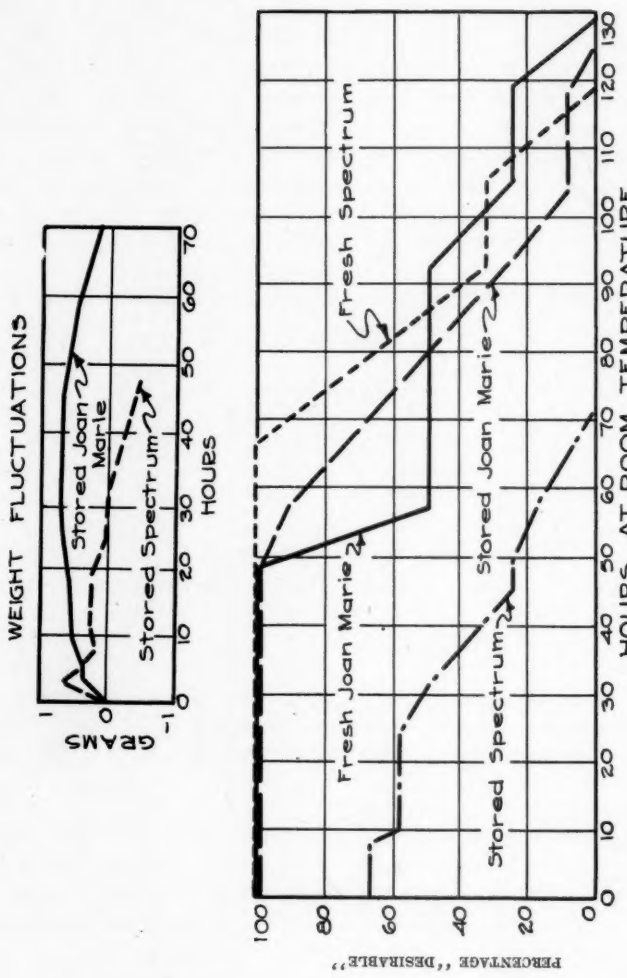

FIG. 5. Carnations stored for 56 days at 33° F. compared with fresh flowers at room temperature. Above, weight changes with time. Below, changes in condition with time.

Fresh Pink Spectrum flowers were superior to Joan Marie, but were much inferior after storage. Pink Spectrum flowers were considerably more advanced in maturity than Joan Marie at the time of storage.

Both kinds of carnations gained in weight in water at room temperature. Joan Marie made a moderate initial gain and steadily gained in weight for 31 hours. This is the normal reaction of fresh carnations. Pink Spectrum reached its maximum in the first few hours and gradually decreased in weight. Except for the first large gain, the Pink Spectrum carnations approximated closely the typical weight losses of flowers stored in water. Their "desirable" length of life at room temperature also paralleled the results with water-stored flowers. The high water absorption in the first few hours accounts for the increased weight of the flowers. More water goes into the hydration of tissues than is transpired.

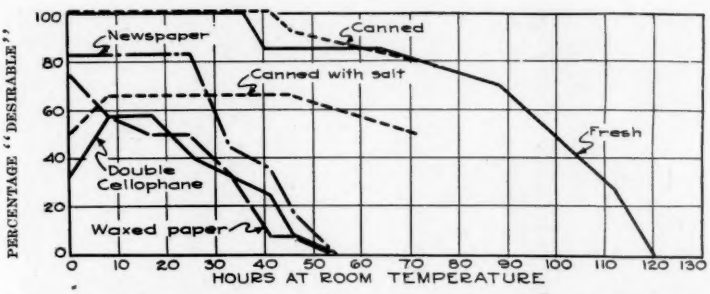

FIG. 6. Results from different methods of "dry pack" storage of carnations. Storage period, 27 days at 34° F.

RELATIONS OF HUMIDITY TO THE KEEPING OF CUT CARNATIONS

HITCHCOCK and ZIMMERMAN (1) found that cut carnations did best in a relative humidity above 98 per cent. The storage of carnations in sealed containers is ideal from the point of view of humidity for in a few hours moisture condenses inside, thus indicating a saturated atmosphere.

Carnations were cut and stored at 34° F. within 2.5 hours after cutting. Lots of flowers were wrapped in newspaper, in waxed tissue paper, and in cellophane; gallon honey cans with tight fitting lids were also used. The flowers were at no time placed in water until removed to room temperature and humidity. The stems were then clipped and the water was changed every day.

The flowers stored in sealed cans kept more successfully (fig. 6) than those wrapped in newspaper, waxed paper (fig. 9, IV), or cellophane (fig. 9, V). The carnations in the can without salt were fully open (fig. 7, VI) and in excellent condition. They compared very favorably with fresh car-

nations when placed at room temperature and humidity. Some of the carnations stored in the can containing salt (fig. 7, III) were badly decomposed; the others were in fair condition. Two additional blossoms became usable after a few hours in water. The object of the salt was to reduce the humidity but both cans contained moisture, indicating a saturated atmosphere. Excessive desiccation injured the wrapped flowers, those in newspaper (fig. 7, II) being the least affected.

It is interesting to compare the carnations stored in a can (fig. 7, VI, and fig. 10) with those stored by other means. Flowers stored in the sealed gallon can were fully open, crisp, and had firm calyces; the others were soft and small, as shown in figure 7. After 22.5 hours in water at room temperature the flowers stored in the gallon can were still superior (fig. 8, IV); those remaining from the can with added salt were equal in quality to

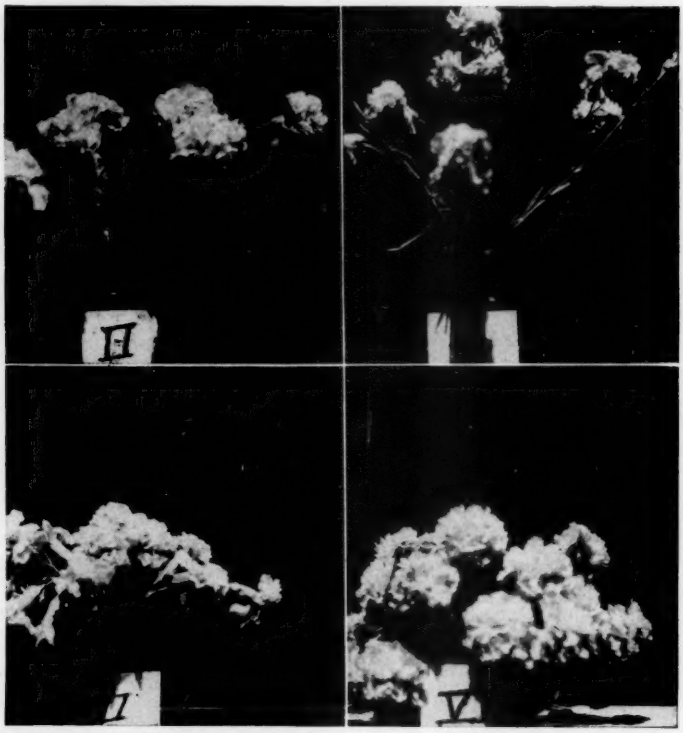

FIG. 7. Condition of carnations at the time of removal (storage period 27 days). II, stored wrapped in newspaper. III, stored in a gallon can with salt. V, stored wrapped in double cellophane. VI, stored in a gallon without salt.

those from the first can. The carnations which had been wrapped and stored in newspapers (fig. 8, II) still lacked the size and firmness of those stored in cans.

Discussion

The successful storage of cut carnations depends upon arresting the normal development of the flowers without initiating undesirable physiological reactions. It is not only necessary that the flowers should look well when removed from storage, but they must hold up satisfactorily at room

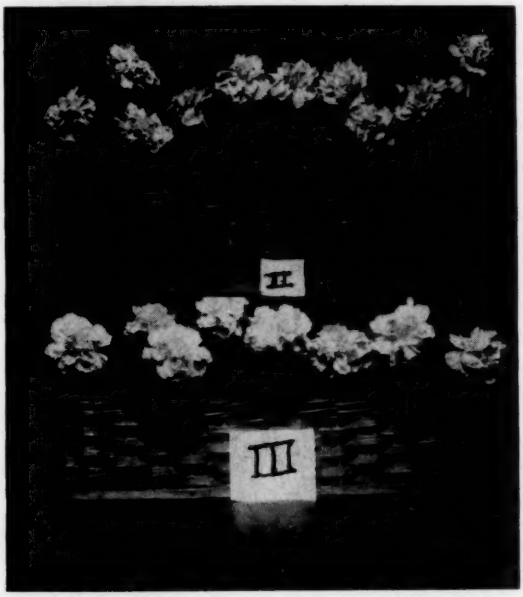

FIG. 8. Condition of carnations after 22.5 hours at room temperature. Only flowers of "desirable" grade were photographed. II, stored wrapped in newspaper. 83 per cent. were "desirable." III, stored in a gallon can with salt. 70 per cent. were "desirable."

temperature. Low temperature is the storage factor upon which most dependence is placed. It has been pointed out, however, that carnations are unfavorably affected by the usual storage at low temperatures. Other variables which may enter into the problem are: the turgidity of the flowers during storage; the humidity of the storage atmosphere and its gas content, particularly the percentage of carbon dioxide and oxygen; and finally the condition and inherent storage qualities of the flowers it is desired to hold.

The importance of low temperatures for long time storage is self-evident.

Both respiration and development toward maturity must be kept at a minimum if the flowers are to survive.

In addition to the use of low temperatures to slow respiration it is probable that more attention should be given to the reserves of the flowers at the time of storage.

The importance of reserves is evidenced by the greatly reduced carbohydrate percentage in flowers stored in water and their corresponding lack in keeping qualities. "Dry pack" carnations, on the other hand, were com-

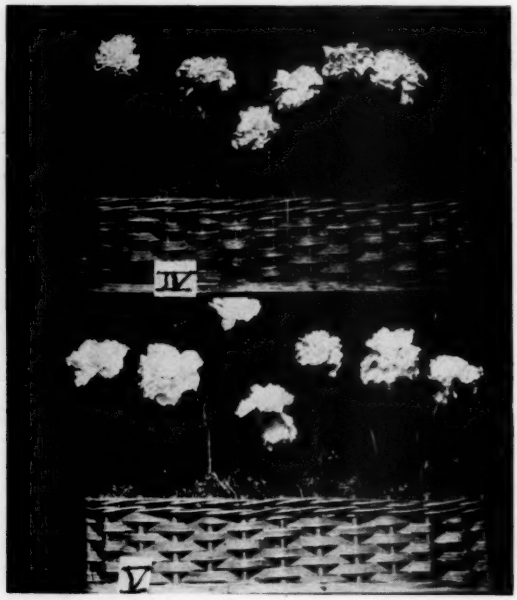

FIG. 9. IV, stored wrapped in waxed paper. 50 per cent. were "desirable" (border-line case). V, stored wrapped in double cellophane. 58 per cent. were "desirable."

parable with fresh flowers as to keeping qualities and food reserves. Prepicking treatments, such as harvesting on the afternoon of a sunny day, and watering lightly for a day or two beforehand, should theoretically increase the storage period.

Turgor as well as temperature has an important rôle in growth and maturity. The experiments show that unlimited moisture during the storage period is generally detrimental to the keeping qualities of cut carnations. PRIESTLEY (4) states that division and elongation of the cells in a growing meristem depend on high moisture content at the growing point. It is not

unexpected, therefore, to find that partly matured carnations opened fully during storage in water, while comparable flowers, dry packed in sealed containers, developed much more slowly and opened very little during the storage period. NEFF and LOOMIS (3) concluded that maturity and old age is the result of cell development. Cell development can progress normally only when there is sufficient moisture present to maintain high turgor pressure. Reducing the turgor pressure, therefore, reduces the rate of maturity. Controlled wilting previous to storage by the "dry pack" method shows considerable promise.

Wrapping flowers in various materials can probably be used to control humidity around the flowers and prevent excessive drying. The correct

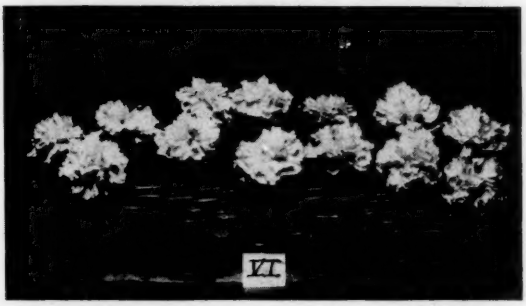

FIG. 10. VI, stored in a gallon can. 100 per cent. were "desirable."

material and number of wrappings could be determined only by trial, and a standardization applicable to all storage rooms and flowers would be impossible. The use of sealed containers reduces the guess work. A few hours after being placed in low temperature, moisture collects on the sides of containers, indicating a saturated atmosphere.

The degree of tightness of the container influences the accumulation of carbon dioxide and reduction of oxygen. For long storage periods an accurate knowledge of the carbon dioxide and oxygen percentages is necessary, otherwise injury, or lack of injury, is a matter of luck. Unquestionably, the benefits derived from carbon dioxide accumulation and oxygen decrease in many of the experiments were offset by the injurious effects of high carbon dioxide and low oxygen present at the end of the experiments. The best results were obtained when the containers were aerated.

The poor keeping qualities of carnations stored in water could not be attributed to lack of ability to absorb water. In only one experiment was there a reduction in rate of water loss from "wet pack" as compared to fresh, or "dry pack" flowers.

Summary

1. Carnations stored at 33° F. were superior to those stored at 40° F.

2. Carnations stored without being placed in water, were comparable, in keeping quality and carbohydrate reserves, with fresh carnations.

3. Carnations stored with the stems in water were inferior in keeping quality, and low in carbohydrates.

4. Fresh and "dry pack" storage carnations increased in weight when placed in water at room temperature. Carnations stored in water lost weight at room temperature. There was a correlation between weight fluctuations and keeping quality.

5. Carnations wilted previous to storing "dry pack" were superior to more turgid "dry pack" carnations.

6. In general, fresh "dry pack" and water stored carnations had the same rate of transpiration.

7. Carnations were favored by a high humidity. Sealed containers produced this condition; wrapped carnations suffered from desiccation.

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APPLICATION OF THE CERIC SULPHATE METHOD IN THE ANALYSIS OF CARBOHYDRATES IN THE ROOTS OF LEPIDIUM AND CONVOLVULUS[1]

C. G. Barr

(WITH FOUR FIGURES)

Introduction

During the course of a general investigation of the carbohydrates in the roots of perennial weeds it was found that copper reduction methods commonly employed for sugar determinations were in some respects unsatisfactory for the analysis of whiteweed, Lepidium. The greatest difficulty was encountered in the determination of the total sugar content from the alcoholic root extract. This experience led to further investigation to discover suitable methods for this work.

The ceric sulphate method adopted is one which has been described (**2**) and shown to be useful in the determination of varying quantities of reducing substances in plant tissue. The writer found it to be workable for estimating moderately large quantities of sugars, especially in the determination of total sugars in Lepidium by invertase hydrolysis. It has the distinct advantage in being useful in experiments involving the analysis of great numbers of samples. The use of ceric sulphate eliminates the steps of precipitating, filtering and measuring reduced copper which were the sources of difficulty encountered in the analysis of the soluble carbohydrates in Lepidium.

Materials and methods

The material used was the roots of *Lepidium draba* var. *repens,* growing in the vicinity of Fort Collins, Colorado. The samples used for the experiments on methods were aliquots from the extracts and portions of the residues of those samples which had been collected for a study of the reserve carbohydrates.

The fresh roots were killed in boiling 95 per cent. alcohol, the soluble carbohydrates extracted by a series of decantations with 80 per cent. alcohol, and the total made to a convenient volume. Aliquot portions of the extract were taken for analysis, the alcohol removed, and the resulting water solutions cleared with neutral lead acetate and made to volume. Deleading was accomplished by filtering into beakers containing anhydrous sodium oxalate. Sodium oxalate, however, cannot be used as a deleading agent when the

[1] Contribution from the Colorado Agricultural Experiment Station in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Published with the approval of the Director of the Experiment Station.

analyses are made with ceric sulphate since the oxalate is oxidized by ceric sulphate. Dry dipotassium phosphate is therefore recommended instead of the sodium oxalate.

No difficulty was encountered when the cleared solution was boiled with Fehling's solution for reducing sugar determinations. Only small quantities of reducing sugars, however, were found to be present. For total sugar determinations 50-ml. samples of the cleared solution were adjusted to the proper pH with 10 per cent. acetic acid and about 6 drops of 1 per cent. solution of invertase scales were added. Extracts containing sucrose are hydrolyzed to invert sugars in about two hours at 20° C. The extracts of bindweed and most other plant tissues are satisfactorily analyzed in this manner for total sugars by the MUNSON-WALKER (**4**) method of copper reduction but in the analysis of Lepidium considerable difficulty was encountered.

Upon heating the hydrolyzed samples of Lepidium with Fehling's solution, the contents of the beaker first appeared dark, then passed through several shades of bluish green and finally became reddish brown by the time the solution boiled. After boiling for two minutes and filtering off the excess Fehling's solution, the precipitate remaining in the Gooch filter was rather dark brown in color and not completely soluble in the ferric ammonium sulphate solution used in the modified BERTRAND (**1**) method for the estimation of copper. Considerable residue always remained in the filter even after breaking up the asbestos mat and treating a number of times with the alum solution. Samples were diluted and tests were run which showed that the peculiar behavior was not due to a high concentration of the sugar, although dilution to some extent reduced the difficulty in filtering the cuprous oxide. Fluctuating values were obtained with potassium permanganate titrations on duplicate samples and frequently different values were obtained from the same cleared and deleaded sample. These results suggested the possibility that some substance might have been left in the samples by the clearing reagent which interfered with copper reduction. Samples were therefore cleared with Horne's dry subacetate, but these samples showed the same reaction. This behavior indicated that the material causing the difficulty in the analysis was probably carbohydrate in nature.

When hydrochloric or sulphuric acid was used for hydrolysis instead of the enzyme, hydrolysis appeared to be complete and the substances contained in the hydrolysate reacted with the Fehling's solution to produce a cuprous oxide precipitate which behaved more normally.

No attempt is made in this paper to present evidence for the identification of the material present causing the difficulty, the object being to show that for sugar analysis of Lepidium a shorter and more convenient method may be used which yields comparable and trustworthy results.

The expense of chemicals and the labor involved in the analysis of plant

tissue by the several copper methods are items of primary importance in cases where funds are limited. This is especially true if the investigation requires the analysis of large numbers of samples for the various carbohydrate fractions.

HASSID (**2**) has shown that ceric sulphate may be used in the determination of varying quantities of sugar in plant tissue. He presented data on five different dry plant materials, and showed that the results by this method were as much as 5 per cent. higher than by the MUNSON-WALKER method.

Preliminary tests of the two methods were made by the writer on leaves of maize and bindweed and also on the roots of bindweed and white weed. Reducing sugar and total sugar values were from 4 to 10 per cent. higher when determined by the ceric sulphate method than by the modified MUNSON-WALKER method. Apparently the variation was dependent upon the tissue under investigation. The results showed closer agreement between the two methods for vegetative tissue than for root material.

As described by HASSID, the principle involves the reduction of alkaline potassium ferricyanide by the reducing substances in the sample and the titration of the reduced iron with a standard ceric sulphate solution. The ceric sulphate is standardized against 0.1 N ferrous sulphate and the glucose factor determined by checking with a carefully prepared 0.1 per cent. solution of pure dextrose.

The solutions used are prepared as follows:

A. Alkaline potassium ferricyanide

4 gm. of potassium ferricyanide and 24 gm. of sodium carbonate are dissolved in distilled water and made up to one liter. This solution is quite stable if kept in the dark in a brown bottle.

B. Ceric sulphate

8.3 gm. of c. p. ceric sulphate are dissolved in about 50 ml. of distilled water in a one liter volumetric flask. To this is added 100 ml. of 1:1 sulphuric acid and made to volume.

To 10 ml. aliquots of the sugar solution in 200-ml. Erlenmeyer flasks, 25 ml. of the alkaline potassium ferricyanide are added. This is heated on a boiling water bath for exactly ten minutes and then cooled in running tap water. The solution is then neutralized with 10 ml. of 5 N H_2SO_4 and titrated with the standard ceric sulphate using about 3 drops of 0.025 M ortho-phenanthroline ferrous sulphate complex as an inside indicator. It was found that the indicator solution may be diluted with distilled water to 50 per cent. of the above concentration and used with satisfactory results. HASSID (**3**) has also shown that Setopaline C is a more suitable indicator than ortho-phenanthroline[2] for the determination of quantities of sugar from 0.3 to 3.50 mg. per sample.

[2] The ceric sulphate and ortho-phenanthroline may be obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio.

A simple calculation gives the sugar content in percentage of green weight. If 0.011626 N ceric sulphate is used, standardizations and tests

TABLE I

CARBOHYDRATES* OF THE WHITE WEED GROWN ON UNCULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON-WALKER METHOD		CERIC SULPHATE METHOD	
	TOTAL SUGARS	TOTAL CARBOHYDRATES	TOTAL SUGARS	TOTAL CARBOHYDRATES
	%	%	%	%
April 25				
(1–2)	0.55	4.66	1.50	6.54
(4–5)	1.35	6.65	2.82	6.59
May 9				
(18–19)	0.96	6.26	1.77	7.74
(20–21)	1.51	6.60	2.26	7.76
May 25				
(55–56)	1.25	11.55	2.15	13.08
(57–58)	1.64	11.69	2.88	12.39
June 8				
(71–72)	1.68	15.55	2.77	24.75
(73–74)	2.30	14.16	3.95	23.39
June 22				
(79–80)	0.95	17.20	1.91	23.18
(81–82)	1.81	16.50	3.00	22.54
July 6				
(95–96)	1.41	21.20	3.76	30.24
(97–98)	2.40	20.00	4.16	27.74
July 21				
(102–103)	1.68	23.12	2.96	31.25
(104–105)	2.40	21.28	3.86	29.30
August 2				
(125–126)	1.54	22.24	2.77	28.75
(127–128)	1.70	22.59	2.88	29.64
August 18				
(147–148)	.....	24.00	2.20	24.25
(149–150)	0.88	22.10	2.99	26.50
Sept. 1				
(159–160)	0.94	21.52	1.98	24.95
(161–162)	1.68	21.94	2.96	26.34
Sept. 13				
(177–178)	1.23	19.70	2.16	22.53
(179–180)	1.35	20.94	2.24	26.64
Sept. 30				
(189–190)	1.72	18.52	2.51	23.57
(191–192)	1.40	20.08	2.04	26.28
Oct. 16				
(201–202)	2.67	17.10	3.49	22.89
(203–204)	2.23	19.60	3.10	24.79
Oct. 29				
(209–210)	4.31	17.40	5.46	25.46
(211–212)	3.19	18.29	4.36	27.05
Dec. 3				
(217–218)	6.34	14.30	7.44	20.22
(219–220)	4.52	15.40	6.65	23.72

* Expressed as percentage of green weight.

against pure glucose show that 2.47 ml. are equivalent to 1 mg. of glucose. One hundred ml. of extract cleared and made to 250 ml. volume represents one-fifth of the original alcoholic extract which contains the total amount of sugars from 50 gm. of fresh tissue. If a 10-ml. aliquot of this cleared solution is used in the sugar determination and a titration of 14.28 ml. of ceric sulphate is obtained after deducting the reagent blank, the per cent. sugar would be calculated as follows:

$$\left(\frac{500}{100}\right)\times\left(\frac{250}{10}\right)\times\left(\frac{14.28}{2.47}\right)\times\left(\frac{100}{50000}\right)=1.44 \text{ per cent.}$$

Solutions containing large quantities of reducing sugars may contain more than 20 mg. of glucose equivalent to the 10 ml. aliquots, in which case the 25 ml. of ferricyanide will not contain sufficient ferric iron. This may be avoided in two ways: (1) by proper dilution of the sugar solution or (2) by making up the ferricyanide solution to contain more iron. The latter method was used in this investigation since there is probably less error involved in this procedure than in further dilution of the sample. When higher sugar values are to be determined the concentration of the ceric sulphate should be correspondingly increased to 0.02 to 0.03 N in order to obtain suitable titration values.

TABLE II

CARBOHYDRATES* OF THE WHITE WEED GROWN ON CULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON-WALKER METHOD		CERIC SULPHATE METHOD	
	TOTAL SUGARS	TOTAL CARBOHYDRATES	TOTAL SUGARS	TOTAL CARBOHYDRATES
	%	%	%	%
May 9				
(22–23)	0.39	5.40	1.38	5.74
(24–25)	1.70	5.50	2.79	8.47
May 25				
(51–52)	0.14	4.03	1.10	6.70
(53–54)	1.30	5.53	2.68	8.77
June 8				
(67–68)	0.22	3.40	1.00	8.84
(69–70)	0.89	3.90	1.60	7.24
June 22				
(75–76)	0.22	3.50	1.12	5.79
(77–78)	0.10	3.40	1.01	6.02
July 6				
(91–92)	0.30	3.60	1.42	7.20
(93–94)	0.40	4.30	1.85	7.52
July 21				
(99–)	0.50	5.40	2.11	9.25
(101–102)	0.50	5.20	2.12	9.08
August 2				
(122–)	0.70	4.36	2.52	8.10
(123–124)	1.98	6.50	3.17	8.33

* Expressed as percentage of green weight.

Samples of Lepidium which had been collected for a study of the trend of root carbohydrates at two-week intervals from April 25 to October 29, 1936, were used also for this study. Data are presented in table I comparing the total sugar values in percentage of green weight as determined by the MUNSON-WALKER method of copper precipitation and by the ceric sulphate method. Each value represents the average of duplicate samples. Tests were made on root samples from the first- and second-foot levels from plants growing on cultivated and undisturbed plots. A total of 220 separate white weed samples were analyzed for sugar content by these two methods. A study of tables I and II shows that the general trend of total sugar is the same when estimated by either of the two methods. The variations are of the same order but somewhat more distinct when calculated by the ceric sulphate method. The higher values and greater variations may be accounted for by assuming that the sample contains certain substances and impurities, such as acetates or citrates, which have a reducing effect upon the ferricyanide.

The total sugar values obtained by the two methods for the samples from uncultivated plots are shown graphically and compared in figure 1.

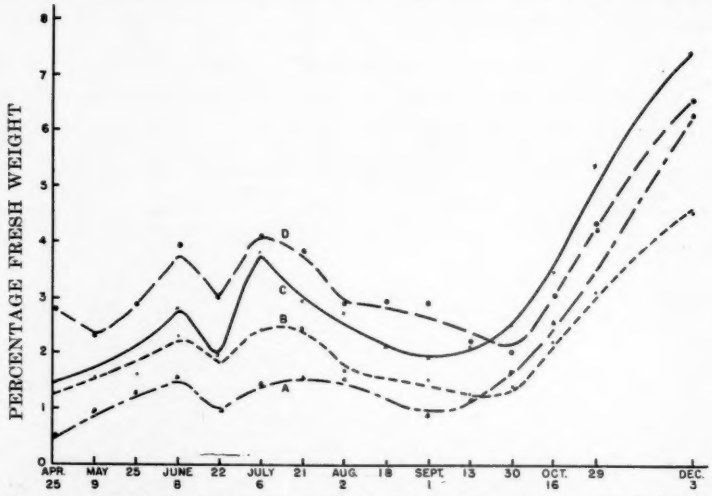

FIG. 1. Comparison of the ceric sulphate and MUNSON-WALKER methods for total sugars in white weed roots. A—First foot level by the MUNSON-WALKER method. B—Second foot level by the MUNSON-WALKER method. C—First foot level by the ceric sulphate method. D—Second foot level by the ceric sulphate method.

It is observed that the curves representing the percentages by the two methods are nearly parallel throughout.

Data are given in tables III and IV for *Convolvulus arvensis* which show

TABLE III

SUGARS* OF THE BINDWEED GROWN ON UNCULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON-WALKER METHOD			CERIC SULPHATE METHOD		
	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE
	%	%	%	%	%	%
April 25 (1–2)	0.55	2.44	1.91	1.24	3.11	1.87
May 9 (11–12)	0.51	1.91	1.40	0.83	2.24	1.41
May 25 (21–22)	0.66	2.25	1.59	1.01	2.52	1.51
June 8 (31–32)	1.07	2.54	1.47	3.90	6.14	4.24
June 22 (35–36)	0.83	2.81	1.98	3.01	4.93	1.91
July 6 (43–44)	0.77	2.66	1.89	1.05	3.03	1.98
July 20 (55–56)	1.31	3.94	2.63	2.04	4.52	2.48
August 1 (63–64)	1.21	3.96	2.75	2.08	4.90	2.82
August 17 (73–74)	1.25	2.79	1.54	2.04	3.46	1.42
August 31 (83–84)	1.56	3.97	2.41	2.32	4.77	2.45
Sept. 16 (93–94)	1.62	4.46	2.84	2.13	5.19	3.06
Sept. 29 (99–100)	1.92	5.77	3.85	2.90	6.94	4.04
Oct. 15 (123–124)	1.67	5.83	4.16	2.05	6.95	4.90
Oct. 30 (127–128)	1.00	7.09	6.09	1.70	7.77	6.07

* Expressed as percentage of green weight.

somewhat closer agreement by the two methods on the total sugar values for this material than for white weed. The curves in figure 2 are so nearly parallel that the trends are identical.

Bindweed roots contain appreciable quantities of reducing sugars and the reducing values obtained by each of the two methods exhibit the same fluctuations. It was mentioned in an earlier part of this paper that bindweed roots behaved normally toward invertase when analyzed for total sugars by the MUNSON-WALKER method of precipitation. Tables III and IV show the sucrose values for bindweed roots by the MUNSON-WALKER and ceric sulphate methods respectively. Comparison of these values shows strikingly close agreement for sucrose by the two methods. There are but two samples in the series which show greater than 0.2 per cent. variation in sucrose by the two methods of analysis. Several additional tests were

TABLE IV

SUGARS* OF BINDWEED GROWN ON CULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON-WALKER METHOD			CERIC SULPHATE METHOD		
	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE
	%	%	%	%	%	%
May 25 (23–24)	0.53	1.50	0.97	0.91	2.12	1.21
June 8 (29–30)	0.40	1.77	1.37	2.00	5.38	3.38
June 22 (33–34)	0.26	1.19	0.93	1.66	4.15	2.49
July 6 (45–46)	0.03	0.82	0.79	0.61	1.18	0.57
July 20 (53–54)	0.01	0.70	0.69	0.41	0.85	0.44
Aug. 1 (61–62)	0.02	0.71	0.69	0.77	1.30	0.53
Aug. 17 (71–72)	.....	0.71	0.71	0.81	1.30	0.49
Aug. 31 (81–82)	0.20	0.98	0.78	0.46	1.13	0.61
Sept. 16 (91–92)	0.17	0.54	0.37	0.46	0.84	0.38
Sept. 29 (101–102)	0.11	0.80	0.69	0.47	1.08	0.61
Oct. 15 (121–122)	.....	0.95	0.95	0.46	1.55	1.09

* Expressed as percentage of green weight.

made on the extract of these samples but each time the results were identical.

By referring to figure 2 it will be observed that approximately the same difference exists between the two methods of analysis for either reducing sugars or total sugars. It has been shown above that calculating the percentage of sucrose as the difference between the total sugars and the reducing sugars yields the same values by either method. Since the same error is present whether analyses are made for total or reducing substances the trend, of course, remains the same. The question arises, however, whether the higher values obtained for reducing substances by the ceric sulphate method actually represent the reducing sugar value or whether the apparent higher sugar value is due to the reducing action of other substances contained in the sample such as citrate and acetate buffers. WHITMOYER (5) reports that chlorides and tartrates have practically no effect upon the reduction of ferricyanide by invert sugar while acetates and citrates slightly affect the reduction.

In order to determine whether or not the higher values were the result of reducing action by substances other than sugars, which could be removed

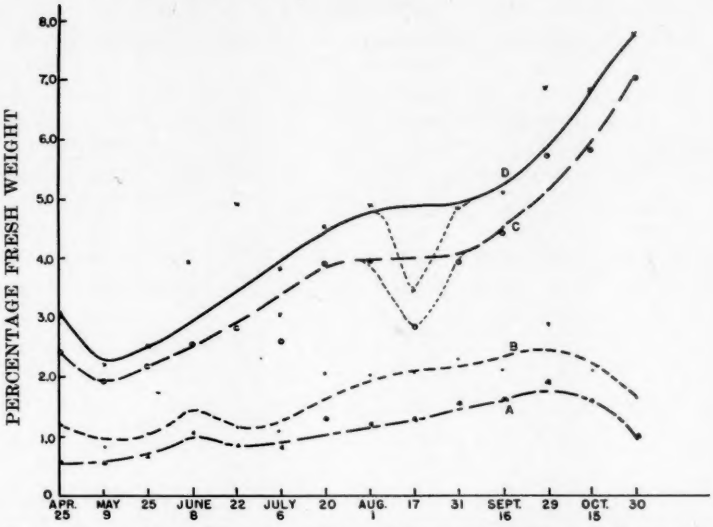

FIG 2. Comparison of the ceric sulphate and MUNSON-WALKER methods for reducing and total sugars in bindweed roots. A—Reducing sugars by the MUNSON-WALKER method. B—Reducing sugars by the ceric sulphate method. C—Total sugars by the MUNSON-WALKER method. D—Total sugars by the ceric sulphate method.

by activated carbon, tests were made on samples treated with carbon in comparison with untreated samples. Data presented in table V give the results

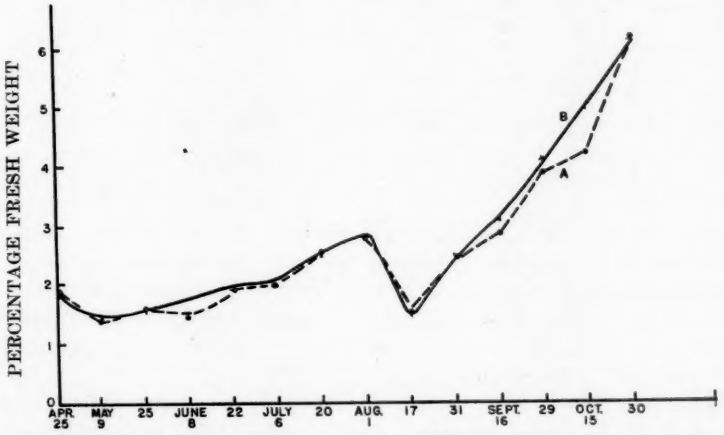

FIG 3. Comparison of the ceric sulphate and MUNSON-WALKER methods for sucrose in bindweed roots. A—Sucrose by the MUNSON-WALKER method. B—Sucrose by the ceric sulphate method.

TABLE V

EFFECT OF VEGETABLE CARBON "NUCHAR" UPON ANALYSES OF PLANT SUGARS AND PURE SUCROSE

SAMPLE NO CARBON	ANALYSIS BY MUNSON-WALKER METHOD				ANALYSIS BY CERIC SULPHATE METHOD			
	TITRATION	NET AVE.	COPPER	DEXTROSE	TITRATION	NET AVE.	DEXTROSE	DEXTROSE PER 50 CC.
	cc.	*cc.*	*mg.*	*mg.*	*cc.*	*cc.*	*mg.*	*mg.*
97	16.80	16.20	102.70	50.20	18.70	18.05	12.45	62.25
98	16.40	.....	.....	.....	18.20	.....	.....	.....
99	18.60	18.00	114.10	56.10	20.00	19.95	13.75	68.75
100	18.20	.....	.....	.....	20.70	.....	.....	.....
107	5.90	5.50	34.90	16.60	6.40	6.00	4.14	20.70
109	2.20	1.80	11.41	5.30	4.90	4.50	3.10	15.50
103	5.15	5.15	31.80	15.10	8.00	6.60	3.55	17.75
104	5.15	.....	.....	.....	7.40	.....	.....	.....
107C	10.70	10.75	66.09	31.95	15.82	14.97	8.05	40.25
108C	10.80	.....	.....	.....	16.33	.....	.....	.....
W110	8.60	8.82	52.98	25.50	16.10	15.00	8.06	40.30
W111	9.05	.....	.....	.....	16.10	.....	.....	.....
Sucrose	23.50	.....	.....	.....	22.30	.....	.....	.....
	23.00	22.70	144.00	71.40	22.00	21.45	15.20	76.00
CARBON TREATED BEFORE INVERTASE HYDROLYSIS								
97	17.10	16.55	105.00	51.50	18.80	17.85	12.31	61.55
98	17.00	.....	.....	.....	17.70	.....	.....	.....
99	18.30	17.70	112.20	55.20	20.40	19.80	13.65	68.25
100	18.10	.....	.....	.....	20.00	.....	.....	.....
107	6.10	5.60	35.50	16.90	7.00	6.60	4.50	22.50
109	3.90	3.40	21.58	10.10	4.60	4.20	2.90	14.50
103	5.90	4.95	30.50	14.40	8.34	6.47	3.48	17.40
104	5.40	.....	.....	.....	6.81	.....	.....	.....
107C	11.00	10.50	64.61	31.30	15.20	14.80	7.96	39.80
108C	11.40	.....	.....	.....	16.60	.....	.....	.....
W110	10.00	9.10	56.05	27.00	15.35	14.55	7.82	39.10
W111	9.60	.....	.....	.....	15.95	.....	.....	.....
Sucrose	23.10	.....	.....	.....	22.00	.....	.....	.....
	23.70	22.80	144.50	71.60	21.70	21.15	15.00	75.00

obtained by the two methods. Although the data are meager, they are sufficient to show that for the samples tested the carbon had some effect on percentage of sugar whether run by the MUNSON-WALKER method or by the ceric sulphate method. Because of the varying results obtained and the large number of samples to be analyzed it was concluded that treatment with carbon would not be practical.

Since ceric sulphate was suitable for the analysis of reducing sugars and sucrose in plant tissue, it seemed reasonable to believe that it could be applied as a measure of polysaccharides which yield reducing substances upon

hydrolysis. Dry samples of the residue were therefore treated with taka diastase, cleared and deleaded, hydrolyzed with 1 + 20 hydrochloric acid, neutralized, and determinations made for reducing substances on aliquot portions with ceric sulphate. The acid hydrolyzable substances were also determined by this method. The results obtained for the polysaccharide fractions were very satisfactory and it is believed that materials which yield reducing substances upon hydrolysis may be determined by the use of ceric sulphate with results comparable to those obtained by copper reduction methods.

The values obtained by the two methods for total carbohydrates in white weed roots are given in tables I and II. The sum of sugars, starch (or diastase extract), and the acid hydrolyzable substances represents the total carbohydrates; these values are shown graphically in figure 4. Although

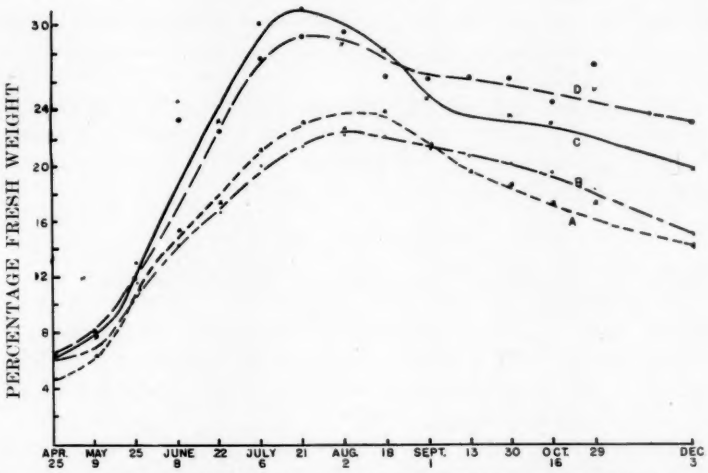

FIG. 4. Comparison of the ceric sulphate and MUNSON-WALKER methods for total carbohydrates in white weed roots. A—First foot level by the MUNSON-WALKER method. B—Second foot level by the MUNSON-WALKER method. C—First foot level by the ceric sulphate method. D—Second foot level by the ceric sulphate method.

values obtained by the ceric sulphate method were decidedly higher, especially when the carbohydrates were at the maximum for the season, the general trend of the carbohydrate content was the same by the two methods. For practical purposes in a weed-control program we believe that estimations of root reserves made with ceric sulphate give a sufficiently clear picture of the carbohydrate content.

Summary

1. The method involving copper reduction was unsuitable for the determination of total sugars in the root extracts of Lepidium (white weed).

2. The method using ceric sulphate to determine carbohydrate content is discussed.

3. Data are presented which were obtained by the use of ceric sulphate in the determination of total sugars and total carbohydrates in the roots of Lepidium.

4. The data show close agreement between the ceric sulphate method and the MUNSON-WALKER method for reducing sugar content of bindweed roots. Reducing sugars and total sugars by ceric sulphate were from 1 to 5 per cent. higher than values obtained by the MUNSON-WALKER method.

5. Sucrose in root extracts of bindweed was determined with equal accuracy by either method.

6. Tests by both methods on samples treated with vegetable carbon in comparison with untreated samples gave varying results which made its use of little value in this investigation.

7. It is suggested that ceric sulphate may be used to obtain reliable values for carbohydrate fractions in root extracts of white weed and bindweed. The method has certain practical advantages: it is rapid, convenient, economical, and trustworthy.

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EFFECT OF HEAVY AND LATE APPLICATIONS OF NITROGENOUS FERTILIZER ON THE COLD RESISTANCE OF KENTUCKY BLUEGRASS

J. C. Carroll and F. A. Welton

Introduction

The conditions under which turf-forming grasses on lawns and recreational fields are maintained are highly exacting. This is due chiefly to frequent and often close defoliation. On golf courses and athletic fields the situation is further aggravated by much trampling. To maintain a good sward under such adverse conditions resort is often made to liberal use of fertilizer. In order to take full advantage of the cool growing weather of late autumn and early spring, top-dressings are often made in the fall.

These applications are regarded as desirable where annual weeds such as crabgrass are a pest. Under such conditions spring and summer treatments may do more harm than good through undue stimulation of the weeds. In the judgment of many practical turf growers, however, late fall applications are detrimental because, in their opinion, they interfere with the normal "hardening off" of grass with the coming of cold weather and thus render it less resistant to the low temperatures of winter.

The purpose of the work reported in this paper was to determine whether or not the above assumption held by certain growers has any basis in fact. The investigation was comprised of two parts. (1) Certain physico-chemical determinations were made on unfertilized and heavily fertilized grass, determinations of constituents which are generally regarded as associated with winter hardiness in wheat. (2) The two classes of grass were exposed to varying degrees of artificial refrigeration and thus was determined, under comparable conditions, the resistance of each to low temperature.

Materials and methods

For use in this study three lots of Kentucky bluegrass were produced: one for chemical analyses, and two for artificial refrigeration.

The grass for chemical analysis was obtained from a group of plots seeded August 15, 1928. On account of the small size of the plots (5 by 10 ft.) it was necessary to save clippings from several plots. Although each received its nitrogen through a different carrier, all were top-dressed to receive nitrogen at the same rate, 2.5 pounds per 1000 sq. ft. Applications at this rate were made three times in 1930: April 11, June 30, and September 15; and three times in 1931: April 21, July 29, and October 14.

In each season the plots were mowed at frequent intervals to maintain good lawn conditions. On account of extreme drouth, especially in 1930, infrequent mowing in midsummer sufficed. In addition to each fertilized plot there was an unfertilized plot of the same size which served as a check.

Supplemental material was obtained from a new seeding of Kentucky bluegrass made in August, 1936. A part of this area was fertilized with ammonium sulphate on September 10 and October 22. Both applications were made at the same rate as in the test already described. Samples from the unfertilized and fertilized areas were gathered from time to time during the late fall.

To test the relative resistance of unfertilized and fertilized Kentucky bluegrass to low temperatures a quantity of each kind was grown in gallon jars in a greenhouse in the winter of 1932–1933. The grass was grown in jars so that it could be transferred without disturbance of the roots to an artificial refrigeration room and there exposed to low temperatures. The jars were seeded at the rate of 5 pounds per 1000 sq. ft. Subirrigation was provided by means of a 0.75-inch glass tube placed at one side of the jar. The lower end rested on a layer of sand which covered the bottom of the jar. Some were fertilized with ammonium sulphate applied at the same rate as in the field tests. The first application was made when the grass was approximately 0.5 inch high; second and third applications were made at intervals of 6 and 12 weeks, respectively. The grass was clipped frequently to simulate lawn conditions and was allowed to develop in the greenhouse for approximately 6 months.

To test further the response of heavily fertilized Kentucky bluegrass to low temperatures samples were also taken from the new seeding made in the summer of 1936.

Samples for chemical analyses were collected from the older plots at irregular intervals during the seasons of 1930 and 1931 and from the new seeding during the late fall of 1936. They were taken between the hours of 10:00 and 11:00 A.M. after the grass was dry. In the fall and early winter it was necessary to use a broom in order to get rid of the heavy dew or frost with which the grass was coated. Immediately after clipping, the samples of grass were taken into the laboratory, ground finely with a food chopper, and thoroughly mixed. Suitable portions were taken for determinations of total moisture, free and bound water, expressible sap, total sugars, and pentosans.

Total moisture was determined on 50-gram duplicates by the tolulene distillation method as modified by SAYRE and MORRIS (**14**). The free water was determined on three 25-gram samples by the calorimetric method first introduced by MÜLLER-THURGAU (**7**), and later improved by THOENES (**17**), ROBINSON (**11**), SAYRE (**13**), and MEYER (**6**). The tissue was frozen at

– 25° C. for at least 3 days and the technique followed was essentially the same as that described by SAYRE (**13**). The bound water was obtained by difference.

Determinations of the sap expressible from fresh tissue were made on 100-gram duplicates. A hydraulic press and press cage of the type described by MEYER (**6**) and a pressure of 5000 pounds per square inch were used. A standard procedure was adopted which consisted of allowing the press cage to drain for 5 minutes and driving out with air the few drops remaining in the tube. Immediately after expression of the sap its specific gravity was determined with a hydrometer and its total solids by a refractometer. Duplicate portions (10 to 20 ml.) were preserved in alcohol for sugar analyses.

The total sugar content of the sap was determined from the alcohol-preserved samples by a modification of the QUISUMBING-THOMAS method (**10**), after the inversion of the sucrose by invertase, and calculated on a fresh weight basis. Pentosans were determined in the dried tissue by the method devised by YOUNGBURG (**19**) and modified by SUMINOKURA and NAKAHARA (**16**). Total nitrogen was determined in the dried tissue by the KJELDAHL-GUNNING method (**5**).

To determine the resistance of grass to cold, artificial refrigeration was employed. The limit of cold may, of course, be ascertained either by exposure of the grass for a definite period at progressively lower temperatures or by exposure at a definite temperature for varying periods of time. The former method was used on the grass grown in the greenhouse. Two jars each of unfertilized and fertilized grass were first placed in a hardening chamber at 0° C. for 10 hours. Although the time allowed for hardening was comparatively short, preliminary tests had shown it to be sufficient to cause an increase in bound water. The grass was then subjected to a definite sub-zero temperature for 8 hours. At the end of this period the minimum soil temperature was determined by means of a thermometer previously inserted in the soil to a depth of 1.5 inches. At the expiration of the period of exposure in the freezing chamber the grass was retransferred to the hardening chamber for 8 hours and thence to the greenhouse.

This procedure was repeated with similar groups of jars, each set being exposed to a lower temperature than the one preceding. Triplicate sets were exposed at each temperature. Observations were made on the stand of grass before, and again 3 weeks after, freezing. On the basis of these the percentage of survival was estimated.

To supplement the results obtained on the greenhouse grass, samples were taken from unfertilized and fertilized grass grown out-of-doors in the summer and fall of 1936. The samples were gathered in the fall on successive dates. The dates were not at regular intervals but were selected to

represent a progressive decline in temperature. The temperatures represented the general average that had prevailed during the preceding 5 days. In general the temperature of the individual days of each group was very uniform. By this procedure the grass was permitted to harden off naturally. In transferring the samples to the refrigerator, therefore, it was not necessary to first place them in a hardening chamber as was the case with those grown in the greenhouse; they were transferred directly to the refrigerator.

The procedure employed to expose the grass grown out-of-doors to artificial refrigeration was as follows. A sufficient number of vitrified gallon jars, approximately 6.5 inches in diameter and 7 inches deep, were first filled with Wooster silt loam, the same type of soil as that on which the grass was growing. The moisture content of this soil closely approximated that of the soil from the plots at the time the samples were taken. The soil was compacted firmly in the jars. This was important in order that the lowering of the temperature of the soil in the jars from the surface downward would proceed as uniformly as possible.

By means of a golf green cup hole digger, approximately 4.25 inches in diameter, a core of soil 4 to 5 inches in depth was removed from the center of each jar and replaced with a sample of sod of like size from the plots. The outer rim of soil in the jar was pressed firmly around the core of sod. The samples were then ready for exposure to low temperature in the refrigerator. On each date of freezing a definite number of cores (8 to 12) were lifted, packed in gallon jars, and placed directly in the refrigerator. A thermometer was inserted in the core to a depth of 1.5 inches to record the soil temperature. In this test the variability in exposure to cold was brought about by subjecting the grass to a constant temperature for different periods of time—the reverse of the process followed with the greenhouse grass. The refrigerator was regulated to maintain a constant air temperature of $-25°$ C. The removal of jars was based on a definite change in soil temperature rather than on exposure for a definite period of time. This was thought to be more accurate since previous experiments had shown that exposure of different jars for equal periods did not necessarily result in an equal lowering of the soil temperature. This was probably caused by variations in moisture content and compactness of the soil. As soon as a definite soil temperature had been reached two jars of both unfertilized and fertilized grass were removed from the refrigerator. Others of both classes were removed from time to time as the soil temperatures became progressively lower until finally all had been taken out. As fast as the jars were removed they were placed in a hardening chamber for 8 hours. At the expiration of that time they were taken to a greenhouse to allow time for recovery. Observations were made on the stand of grass before, and again

3 weeks after freezing. On the basis of these, the percentage of survival was estimated.

Experimental results

In a study of this nature several bases on which the results may be interpreted present themselves. In the case of bound water, for example, early investigators chose to express it as percentage of the total moisture content. More recently other workers have expressed it in terms of weight of bound water per unit of dry matter. Again it might be expressed in terms of weight per unit of fresh tissue. With plant materials in which the content of moisture is considerably greater than that of dry matter, it is

TABLE I

EFFECT OF NITROGENOUS FERTILIZER* ON THE NITROGEN AND MOISTURE CONTENT OF KENTUCKY BLUEGRASS

DATE	TOTAL NITROGEN		TOTAL MOISTURE		FREE WATER		BOUND WATER	
	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N
	%	%	%	%	%	%	%	%
1930								
May 29	0.86	1.21	65.4	73.3	37.8	50.9	27.6	22.4
June 12	0.77	1.19	67.3	69.3	43.4	44.4	23.9	24.9
" 26	0.77	1.11	69.6	73.0	48.4	54.5	21.2	18.5
July 3	0.81	1.40	60.4	68.2	44.7	51.8	15.7	16.4
" 16	1.12	1.83	58.0	58.2	41.4	41.1	16.6	17.1
Sept. 3	0.99	1.11	76.8	78.0	57.9	64.2	18.9	13.8
" 10	0.73	1.31	68.7	72.4	52.2	58.2	16.5	14.2
" 18	1.10	1.33	67.6	73.6	53.6	55.9	14.0	17.7
" 26	0.97	1.11	69.6	78.6	52.2	67.2	17.4	11.4
Oct. 7	0.92	1.33	66.4	73.2	49.0	60.8	17.4	12.4
" 20	0.92	1.30	66.8	74.8	58.6	61.1	18.2	13.7
Nov. 4	0.86	1.24	64.0	72.2	44.0	55.0	20.0	17.2
Jan. 6, 1931	.....	.....	63.0	69.4	44.8	54.4	18.2	15.0
1931								
May 15	0.72	0.98	77.4	80.8	59.0	63.4	18.4	17.4
" 29	0.65	0.88	74.6	79.1	55.0	63.9	19.6	15.2
June 15	0.84	1.00	72.3	77.4	59.4	66.4	12.9	11.0
July 2	0.96	1.05	72.5	74.5	58.5	56.6	14.0	17.9
Sept. 16	0.65	0.99	71.5	77.1	60.5	59.6	11.0	17.5
" 25	0.84	0.93	77.2	81.5	58.6	63.6	18.6	17.9
Oct. 13	0.87	1.04	75.8	77.6	54.4	57.4	21.4	20.2
" 21	0.82	1.05	73.2	75.2	52.8	56.9	20.4	18.3
Nov. 3	0.86	1.24	74.8	76.1	52.9	59.8	21.9	16.3
" 28	.....	.....	70.6	75.6	53.6	62.8	17.0	12.8
Jan. 7, 1932	1.21	1.32	67.7	68.9	48.9	52.2	18.8	16.7
1936								
Oct. 20	1.12	1.25	76.4	77.7	69.2	68.7	7.2	7.0
Nov. 12	1.20	1.60	67.0	67.8	57.5	60.8	9.5	7.0
" 16	1.29	1.38	63.4	71.4	53.2	63.0	10.2	8.4
Dec. 1	1.34	1.49	50.0	66.0	43.8	57.0	11.8	9.0

* In this and in subsequent tables the results from the unfertilized and fertilized grass are recorded under the headings of "LOW N" and "HIGH N," respectively.

the opinion of the writers that the third method is to be preferred; it probably presents a clearer picture of the inner conditions of the living plant than either of the preceding. Accordingly, in this study the bound water and other constituents determined are expressed in terms of weight per unit of fresh tissue. The percentages of total nitrogen, total moisture, free water, and bound water found on the various dates of sampling in 1930, 1931, and 1936 are shown in table I.

From table I it is seen that the addition of nitrogenous fertilizer resulted in an increase in the content of total nitrogen on every date of sampling in each of the 3 years. Considered collectively for the 3 years, the increase amounted to approximately 30 per cent. The nitrogenous fertilizer resulted also in an increase in the content of total moisture on all of the dates

TABLE II

EFFECT OF NITROGENOUS FERTILIZER ON CERTAIN PHYSICOCHEMICAL CONSTITUENTS OF KENTUCKY BLUEGRASS

DATE	EXPRESSIBLE SAP		HYDRATION		TOTAL SUGARS		PENTOSANS (ARABINOSE)	
	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N
	%	%	%	%	%	%	%	%
1930								
May 29	26.7	29.0	149	178	2.07	1.92	3.34	1.58
June 12	22.0	25.0	145	154	1.70	2.14	.....	.....
" 26	31.9	40.5	135	133	1.29	1.06	.....	.....
July 3	31.0	32.9	84	122	2.48	1.81	2.77	2.82
" 16	16.1	19.2	105	99	2.43	2.52	.....	.....
Sept. 3	37.3	38.3	181	193	1.57	1.07	.....	.....
" 10	23.2	25.3	125	170	.....	1.97	2.90	2.38
" 18	23.1	25.6	146	193	2.98	1.95	.....	.....
" 26	27.2	39.8	138	194	1.81	1.25	.....	.....
Oct. 7	25.6	30.2	131	172	2.14	1.35	.....	.....
" 20	26.4	27.7	132	203	4.69	3.86	5.10	2.65
Nov. 4	17.9	21.5	135	193	4.45	4.34	6.13	3.87
Jan. 6, 1931	4.5	12.9	160	191	4.82	4.48	.....	.....
1931								
May 15	40.9	43.6	177	215	1.82	1.48	2.32	1.57
" 29	47.0	41.7	123	194	1.31	1.03	.....	.....
June 15	42.4	40.9	119	175	1.36	1.36	.....	.....
July 2	33.0	32.1	153	176	1.22	1.25	.....	.....
Sept. 16	22.3	29.2	177	219	.....	.....	3.46	1.26
" 25	15.8	42.3	275	220	0.78	0.78	.....	.....
Oct. 13	.....	.....	.....	.....	2.94	1.88	2.42	2.01
" 21	18.4	20.2	212	230	3.50	2.41	2.38	1.99
Nov. 3	10.2	13.6	262	268	4.13	1.65	2.90	2.41
Jan. 7, 1932	6.2	10.6	193	193	4.56	2.09	3.70	3.11
1936								
Oct. 20	25.5	30.0	227	272	2.25	2.00	1.92	1.82
Nov. 12	9.8	12.2	179	181	3.58	3.61	2.64	2.02
" 16	14.5	18.9	140	193	5.34	3.57	3.42	2.00
Dec. 1	0.0	7.5	133	177	5.50	3.92	6.62	3.09

of sampling. On the whole the increase amounted to approximately 7 per cent. Most of the increased moisture was in the form of free, or freezable water, since the free water was usually greater in the high- than in the low-nitrogen grass. On the other hand, the bound water was generally lower in the high- than in the low-nitrogen grass and no exceptions occurred in the fall after the coming of cool weather.

The percentages of expressible sap, hydration, total sugars, and pentosans found on each day of sampling in the 3 years, 1930, 1931, and 1936, were as shown in table II.

From table II it is seen that the percentage of expressible sap was almost always higher in the high- than in the low-nitrogen grass. The difference was usually greater in the cool weather of fall than in midsummer. In one year, 1931, it was actually less for a time in the summer. Likewise, the percentage of hydration was, with few exceptions, higher in the high- than in the low-nitrogen grass. On the other hand, the total sugars and pentosans were generally higher in the low- than in the high-nitrogen grass.

The response of the low- and high-nitrogen grass to low temperatures is shown by the refrigeration tests. As previously stated, variability in exposure of grass grown in the greenhouse was effected by changing not the time of exposure but the temperature. The air temperatures to which the grass was exposed, the lowered temperature of the soil, and the percentage of survival are shown in table III.

TABLE III

RESULTS OF FREEZING KENTUCKY BLUEGRASS GROWN IN A GREENHOUSE

AIR TEMPERATURE	FINAL SOIL TEMPERATURE	SURVIVAL AFTER 2 WEEKS	
		LOW N	HIGH N
° C.	° C.	%	%
−12	− 6	95	50
−14	−11	80	45
−16	−14	10	5
−18	−16	10	0

It is evident that the high-nitrogen was more susceptible than low-nitrogen grass to low temperatures since at each sub-zero temperature the percentage of survival was lower. Moreover, the lower the temperature to which the air and soil descended the less was the survival of both classes of grass.

To supplement the results obtained on the greenhouse grass, samples were collected from unfertilized and fertilized grass grown out-of-doors. The date of sampling (accompanied by the hardening temperatures), the scale of descending soil temperatures used, and the percentage of survival

TABLE IV

RESULTS OF FREEZING KENTUCKY BLUEGRASS GROWN OUT-OF-DOORS

FINAL SOIL TEMPERATURE AFTER EXPOSURE FOR VARIOUS PERIODS IN REFRIGERATOR	DATE OF SAMPLING—1936											
	OCTOBER 20 (+6.2° C.)*			NOVEMBER 12 (−2.2° C.)*			NOVEMBER 16 (−2.6° C.)*			DECEMBER 1 (−9.6° C.)*		
	EXPOSURE AT −25° C.	SURVIVAL		EXPOSURE AT −25° C.	SURVIVAL		EXPOSURE AT −25° C.	SURVIVAL		EXPOSURE AT −25° C.	SURVIVAL	
		LOW N	HIGH N		LOW N	HIGH N		LOW N	HIGH N		LOW N	HIGH N
°C.	hr.	%	%	hr.	%	%	hr.	%	%	hr.	%	%
0°	2.5	100	100	2.25	100	100	.....	.....	.....	.....	.....	.....
− 1°	4.0	50	50	4.5	100	75	2.0	100	100	.....	.....	.....
− 3°	5.0	0	0	.....	.....	.....	3.0	100	50	2.75	100	75
− 5°	5.25	0	0	5.0	75	5	3.0	100	10	3.0	100	45
− 7°	.....	.....	.....	.....	.....	.....	3.5	85	5	3.5	95	25
− 9°	.....	.....	.....	5.25	25	0	4.0	80	0	4.0	80	10
−11°	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.25	60	5
−13°	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.0	40	5

* The figures in parentheses accompanying each date of sampling represent the average minimum air temperature for 5 days previous to sampling and, hence, the hardening temperatures to which the grass had been exposed at the time of cutting.

of the low- and high-nitrogen grass are shown in table IV. These results indicate no difference in the resistance of low- and high-nitrogen grass on the first date of sampling. The absence of such difference was probably due to the lack of hardening, since investigators (**1, 2, 18**) have shown that most plants do not begin to harden until the temperature descends to approximately + 6° C. Prior to October 20, 1936, the minimum temperature had seldom descended to that point. Moreover, the physico-chemical composition of the grass shown in tables I and II for 1936 failed to show important differences at that time. From November 4, 1936, until after the last sampling date of December 1, the temperatures were all well below the threshold for hardening, usually below 0° C. The maximum temperatures for a majority of the days were likewise within the hardening range. Further examination of table IV shows that the resistance to cold of both the low- and high-nitrogen grass becomes progressively greater as the hardening temperatures lower. The resistance of the low-nitrogen grass, however, was markedly higher than was that of the high-nitrogen grass. It is pertinent to note that the lower the temperature at the time of sampling the lower the temperature of the soil before killing occurred.

Discussion

Artificial refrigeration of Kentucky bluegrass grown in a greenhouse and hardened in a low temperature chamber showed that high- was more susceptible than low-nitrogen grass to sub-zero temperatures. Likewise, artificial refrigeration of Kentucky bluegrass grown out-of-doors and hardened naturally by gradually lowering temperatures during the fall months, showed that high- was more susceptible than low-nitrogen grass to sub-zero temperatures. In both cases the relatively high nitrogen content of the high-nitrogen grass was induced by heavy and repeated applications of ammonium sulphate (2.5 pounds N per 1000 sq. ft.). The top-dressings on grass grown out-of-doors were made in September and October. The refrigeration tests, therefore, indicate that heavy and late applications of nitrogenous fertilizer to Kentucky bluegrass reduce its resistance to sub-zero temperatures.

The temperatures at which the grass succumbed were higher than those to which it is exposed in most winters in this latitude. In nature, however, it is probable that grass would offer more resistance than in the highly artificial conditions under which it was exposed in these tests. Inasmuch as both classes of grass were exposed under the same conditions the results are comparable and, therefore, represent the relative resistance of the two classes of grass.

Some indication of the reason for the relatively low resistance of the high- as contrasted with the low-nitrogen grass is afforded by the physico-

chemical results shown in tables I and II. Unfortunately, only the grass sampled in 1936 was grown on the same plots and under the same conditions as that which was exposed to low temperatures. Inasmuch as the greenhouse grass was grown on soil of the same type and of equal fertility, and was fertilized with the same frequency and with the same quantity of nitrogen as was that grown out-of-doors, it is probable that the internal responses were similar; therefore, its physico-chemical composition was practically the same as that grown out-of-doors. Assuming this to be the case, the results indicate that applications of nitrogenous fertilizer in the fall, when the grass is naturally "hardening off," reduce the elaboration of hydrophilic colloids. The expressible sap during that period is consistently higher in the high- than in the low-nitrogen grass. On the contrary, the bound water is consistently lower. These findings are in agreement with those of NEWTON (**8, 9**) and MARTIN (**4**), who reported similar differences between non-hardy and hardy varieties of wheat.

The percentage of hydration, that is, the residual water content after expressing the sap (expressed as a percentage of the dry weight), seemingly presents a theoretical contradiction to the conclusions drawn from the relative weights of expressed sap. NEWTON (**8**), however, concluded that it was a less dependable measure of hardiness than relative amounts of sap expressed.

Among the hydrophilic colloids which have been considered by a number of workers (**3, 12, 15**) to play a prominent part in hardiness are the pentosans. The determinations made in this study show that the applications of nitrogen decrease the formation of pentosans in Kentucky bluegrass. The results of these determinations are reported as pentose, since a study of the optical properties[1] of the phenylosazone derivative of the products of acid hydrolysis of the dried tissue proved it to be arabinose. Analyses made on scattered samples showed that only 1 to 2 per cent. of the pentose present was contained in the expressed sap. This is to be expected since such material is found chiefly in the cell wall.

The results also show that with the advent of colder weather there is an increase in the total sugar content of both the low- and high-nitrogen grass. In the low-nitrogen grass, however, the increase is significantly greater. The exact rôle that an increased sugar content plays in the cold resistance of plants is not definitely known. The osmotic pressure of the cell sap is increased and this added pressure increases the resistance of the cell to the withdrawal of water during freezing. The fact, however, that the sugars exert a protective action against frost precipitation of the proteins is probably more significant.

[1] The authors are indebted to Dr. V. H. MORRIS of the Ohio Agricultural Experiment Station for this identification.

Summary

1. Heavy and late applications of nitrogenous fertilizer to Kentucky bluegrass increased the content of total nitrogen and total moisture on every date of sampling, which included two full seasons and a part of a third.

2. The same treatment resulted generally in a higher content of free water, expressible sap, and a higher percentage of hydration in the high- than in the low-nitrogen grass. Usually the differences were less in the summer than in the fall after the coming of cold weather.

3. The same treatment resulted generally in a lower content of bound water, total sugars, and pentosans in the high- than in the low-nitrogen grass. Usually the differences were much accentuated with the coming of cool weather in the fall.

4. Artificial refrigeration of both classes of grass in the unhardened condition showed no difference in resistance to cold.

5. Artificial refrigeration of both classes of grass after hardening showed that the high-nitrogen grass was less resistant than the low-nitrogen grass to low temperatures.

6. Inasmuch as the difference in nitrogen content of the Kentucky bluegrass was induced by heavy and late applications of nitrogenous fertilizer, it follows that such a practice may be expected to lessen somewhat the resistance of Kentucky bluegrass to low temperatures.

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SUGGESTIONS FOR THE USE OF WARBURG RESPIROMETERS IN PLANT PHYSIOLOGICAL INVESTIGATIONS[1]

JAMES W. BROWN

(WITH TWO FIGURES)

Introduction

Although Warburg respirometers have been used extensively in animal physiology for a number of years they have not been employed by plant physiologists as much as their accuracy and convenience seem to warrant. HARRINGTON (**2**) used a modification of the Warburg principle in his respirometers, but TANG (**6**) and MURLIN (**5**) apparently are the only workers to use the standard type of respirometer for studies of seed respiration. The Warburg respirometer permits simultaneous measurement of O_2 consumption and CO_2 production on small samples for short time intervals. This makes possible easy and rapid replication of determinations without excessive use of material. By the use of respirometer vessels of suitable size and shape not only seeds, but small fruits, small seedlings, excised roots, buds, flowers and other plant parts may be placed in them.

A careful study of the technique was made in connection with their use in an investigation of the respiration of acorns at various temperatures. It was found that several factors must be considered which are not included in the technique as given in the papers describing their operation (**4, 5, 6, 7**). This paper suggests certain modifications of the standard procedure together with suggestions on the operation of both large and small respirometer vessels.

Experimental methods

The usual procedure for obtaining concurrent measurements of O_2 consumption and CO_2 production is as follows. The time periods given are those which the writer found necessary for the conditions of his investigation and the letters refer to the sample record sheet at the end of the paper. For the most efficient operation of the respirometers it is desirable to determine experimentally the time periods required for temperature adjustment. This can be done by placing the vessels in the bath under the conditions of the experiment and observing the time necessary to bring the manometric fluid to a constant level after the stopcocks of the manometers are closed.

An acid, usually HCl, is placed in the side arm of the respirometer vessel which is then stoppered. An alkali, usually NaOH or KOH, is placed in the bottom of the vessel or in the central cup if one is present, the support

[1] No doubt some investigators have used most of the suggestions offered in this paper, but since they have never been published in any collected form, they are presented here mainly for the consideration of those who are just beginning to use the method.

for the respiring material is placed in position, the top of the vessel seated and the whole unit attached to the manometer. The apparatus is set upon a shaker so that the vessels are submerged in a constant temperature bath. After the vessel has come to the temperature of the bath (7 minutes) the apparatus is removed from the shaker, taken apart, the respiring material placed in the vessel upon the support, and the unit reassembled and returned to the shaker. The time a is recorded when the material is placed in the vessel, and this time is used as the start of the period of CO_2 production. Three minutes later, after respirometer and material are at the temperature of the bath, the manometric fluid is levelled to the 150-mm. mark in the arm connected to the vessel and the level in the open arm is recorded, b, with the time c, and the stopcock is closed. The time c marks the start of the period of O_2 consumption. The apparatus is left on the shaker for the length of the experiment (30 minutes). The shaker was operated at 70 complete oscillations per minute with a throw of 7 cm. Whenever a reading is made care must be taken to have the manometers in a vertical position.

When the period of O_2 consumption is ended, the time d is recorded and the manometric fluid is again brought to the 150-mm. mark and the open arm reading e is made. The length of the O_2 consumption period is then $d-c$, while the change in manometric graduations, $b-e$, represents the amount of O_2 consumption. The apparatus is taken from the shaker immediately after recording d and e and turned in such a position as to cause the acid from the side arm to flow into the alkali in the vessel and liberate the CO_2 which has been absorbed by the alkali. An excess of acid is necessary and where very small amounts of the reagents are used it is advisable to tilt the unit so that the alkali flows into the side arm and then allow the mixture to return to the vessel to insure more thorough mixing of all of the reagents. The apparatus is then replaced on the shaker and after a period of three minutes the level of the fluid in the closed arm of the manometer is brought again to the 150-mm. mark and a reading of the level in the open arm, f, is recorded with the time g. The time g marks the end of the period of CO_2 production, $g-a$, and the "apparent" amount (see corrections 1 and 3) of CO_2 produced is represented as the change in level of $f-e$ of experimental respirometer minus $f-e$ of the thermobarometer which is substituted for h in the following formula. The volume of the respiring material is determined and allowance for this volume is made in the formula. At the end of the experiment the dry weight of the material is determined if practicable in order to place the results on a dry weight basis.

Whenever a series of determinations is to be made a control respirometer (thermobarometer, check, or blank) must be subjected to the same conditions, reagents, and treatment as the series except that it contains no respir-

ing material. This provides a correction for any temperature and external pressure changes during the run, and for any CO_2 which may be dissolved in the reagents before the experiment.

The following formula is used to calculate the volumes of gas absorbed and produced by the respiring material.

$$x = h\left[\frac{Vg\frac{273}{T} + V_f(\alpha)}{P_o}\right]$$

where:

x = mm.^{3} of gas at S. T. P.
h = height change in manometric graduations, $b - e$.
V_g = free volume of gas in the vessel and manometer to the level of manometric fluid (total volume of apparatus less the volume of the sample, liquids and detachable supports placed in the vessel). See DIXON (**1**).
T = absolute temperature of the water bath surrounding the vessel.
V_f = the volume of all liquids in which the measured gas might dissolve.
α = the solubility of the gas being measured in the contained liquids at the temperature T. BUNSEN'S solubility table has been recommended (**4**), $V_f(\alpha)$ gives the volume of the dissolved gas and this is added to the free gas to give the total volume (this expression was omitted by the writer for reasons presented later in this paper).
P_o = normal pressure in terms of manometric fluid (for BRODIE'S solution, 10,000 mm.).

In experiments where a constant volume of material is to be used at constant temperature, the quantity within the brackets in the above formula remains constant for a given vessel and can be assigned a value which may be referred to as the vessel constant. The vessels used in the present work

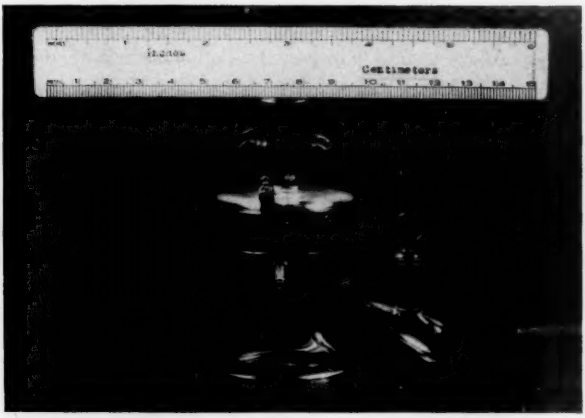

FIG. 1. Modified Warburg respirometer having a volume of approximately 90 ml.

had a capacity of about 90 ml. (fig. 1) and vessel constants of from 7 to 9 whereas the majority of vessels described in the literature are of about 15-ml. volume and have correspondingly smaller vessel constants of about 1 to 3. Where the vessel constant of an apparatus is 1 the reading of a change in pressure on the manometer may be transferred directly to terms of volume; but as the vessel constant increases, the change in pressure as shown by the manometer is smaller by the reciprocal of the vessel constant. When a vessel constant becomes as large as 10, the accuracy with which the reading may be interpreted becomes 0.1 as great as if the vessel constant were 1, or each manometric graduation would represent an internal change of 10 $mm.^3$, whereas with a vessel constant of 1 it represents an internal change of 1 $mm.^3$

The use of the large vessels has made evident certain factors that apparently have frequently been overlooked in calculating the results of determinations made with the smaller vessels. Some of these factors are equally important, however, for small and large vessels.

Correction for interference of O_2 consumption with CO_2 production readings

In previous investigations involving the simultaneous determination of O_2 absorption and CO_2 production with the Warburg apparatus the fact apparently has been overlooked that these two processes are continuously concurrent. The usual technique (**4, 5, 6**) does not measure both processes for the same period of time.

During most of a determination all CO_2 produced is absorbed by alkali in the bottom of the vessels so any change of volume is caused by consumption of O_2 by the respiring material. At the end of this period the manometer is read and the acid in the side arm mixed with the alkali to release the absorbed CO_2. After several minutes the manometer is read again and all change in pressure is attributed to the CO_2 produced by respiration and that already in the reagents. Oxygen consumption, however, has continued during this period so in reality the change in pressure represents that produced by CO_2 minus the O_2 absorbed during the period required for mixing the reagents and again bringing the temperature of the vessels into equilibrium with that of the bath. It is therefore quite evident that in order to obtain the actual volume of CO_2 produced the volume of O_2 absorbed during the period of mixing and temperature adjustment must be added to the apparent volume of CO_2 and the thermobarometer correction applied. This is particularly important where a low R. Q. (respiratory quotient $= \frac{CO_2)}{O_2)}$ exists and also where the time interval is relatively long. Under ordinary conditions the time interval required for mixing reagents and returning the

vessels to the temperature of the bath is 3 to 5 minutes which is 10 to 15 per cent. of the time for which O_2 consumption is being measured. The situation is represented diagramatically in figure 2. The manometer read-

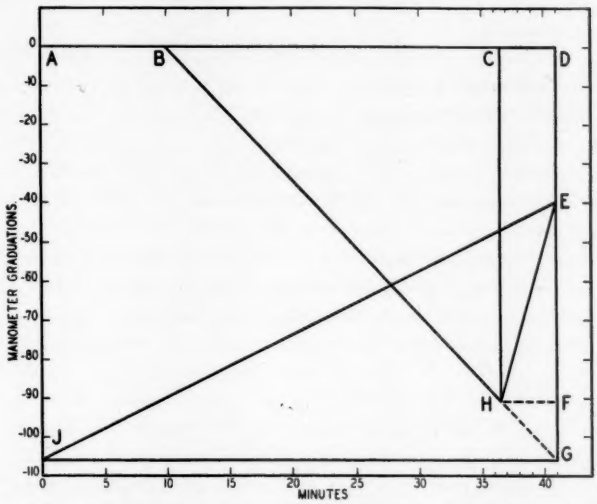

FIG. 2. Suggested procedure compared with usual procedure for obtaining a true reading of CO_2 production.

BH—Progress of O_2 consumption during experiment. Measured at H in 26.5 minutes (BC) by line CH.
H—Point at which reagents were mixed to liberate CO_2. Used by most workers as base for obtaining CO_2 readings.
E—Point at which CO_2 production is measured by difference (vertical or EF) between basic reading at H and final reading at E.
EF—CO_2 production for 41 minutes measured by generally accepted method.
HG—Extrapolation of BH showing estimated progress of O_2 consumption while waiting for reading of CO_2 production at E.
G—Point O_2 consumption is estimated to have reached at time of reading E.
FG—Additional O_2 consumption while waiting to make reading E. Should be added to EF to make correct CO_2 reading.
G—Base which should be used instead of H for correct CO_2 readings.
JG—Length of time CO_2 production is measured. Same as AD. Should be used as base line (zero point) for measuring CO_2 produced during the length of the experiment.
JE—Progress of CO_2 production. When used in conjunction with JG it is possible to make readings of CO_2 at any point during experiment.
BG—Progress of O_2 consumption which makes possible a determination of O_2 consumed at any time during the experiment.

ing for O_2 consumed (fig. 2) in 26.5 minutes is 91 (CH) or approximately 141 for 41 minutes. By the usual method the manometer reading for CO_2 production is 51 (EF) for 41 minutes but by the suggested procedure becomes 66.3 for the same period. Disregarding the solubility of these gases for reasons presented later, the R. Q.'s differ as follows:

$$\frac{51}{141} = 0.36 \text{ R. Q. by the usual procedure}$$

$$\frac{66.3}{141} = 0.47 \text{ R. Q. by the suggested procedure}$$

Correction for solubility of gases

A fault of the usual technique exists in the method of correcting for the solubility of gases in the reagents. Theoretically, the solubility of O_2 and CO_2 in the reagents should be considered, but in most experiments it is a negligible factor and correction for solubility by the usual method may actually decrease the accuracy of the measurements. The usual procedure suggests the use of Bunsen's solubility tables (3) but these tables represent determinations using distilled water as the solvent and the gas at 760 mm. of pressure. Neither of these conditions holds for the respiration experiments, for the final solution in the respirometer is a salt solution in which gases are less soluble than in pure water and the concentration of gases, especially CO_2 is low. In the writer's experiments the concentration of CO_2 in the vessels did not exceed three times its concentration in the normal atmosphere and its pressure would be only about 0.76 mm. or 0.1 per cent. of that called for by standard solubility tables. The alkali used by the writer for absorption of CO_2 was 1.5 ml. of 2N NaOH and the acid used to liberate the CO_2 was 0.6 ml. of 6N HCl. Actually there was much more CO_2 in the alkali before it was placed in the vessels than in the salt solution at the end of a determination, hence none of that produced in respiration could be lost by absorption in the reagents.

Both reagents have been exposed to the O_2 of the air hence are in equilibrium with the concentration of O_2 existing in the vessels at the beginning of the experiment. Since only a very slight change in O_2 pressure occurs during an ordinary determination, solubility of O_2 may also be safely disregarded.

If the vessel constants are corrected for solubility by using the standard solubility tables they are increased. Thus, the product of the vessel constant and the manometric reading is also increased. It happens that the extent of the increase of CO_2 production corrected on the basis of the solubility tables in the usual procedure approaches the increase obtained by the suggested procedure (fig. 2) and the final volume of CO_2 found by both methods may be nearly equal in some cases. The O_2 reading, however, is invariably higher when the solubility tables are used in the usual procedure. The attempt to obtain more accurate results by correcting for solubility of gases by these tables, therefore, actually decreases their accuracy. For these reasons the writer did not use the expression $V_f(\alpha)$ in the formula presented earlier.

Correction for difference between volumes of thermobarometer and other vessels

In making corrections for pressure changes in the vessels as indicated by the behavior of the thermobarometer certain additional corrections are necessary.

During the period in which O_2 absorption is measured there is no change in the mass and volume of gas contained in the thermobarometer even though it may be subjected to temperature and pressure changes. This situation holds for any empty respirometer. The manometric change caused by external conditions for a period of time will be equal in all respirometers regardless of size and content. This equality is evident when the respirometers are run empty but it still exists when they contain respiring material. The thermobarometer correction for pressure and temperature changes during the period of O_2 consumption can therefore be applied directly to the determination of changes in O_2 volume of all respirometers. As the temperature decreases, or the external pressure increases, the manometric reading of the respirometers will decrease; and as the temperature increases or the external pressure decreases the manometric reading of the respirometers will increase. Thus, the thermobarometer correction may be applied by either subtracting the decrease of the thermobarometer reading from, or adding the increase of the thermobarometer reading to, the reading of each of the experimental respirometers.

The correction for CO_2 contained in the reagents cannot be applied directly to the other respirometers, however, unless they all contain equal volumes of gas, because the introduction of equal volumes of gas into systems of different volumes will produce different changes in pressure. If, for example, the total volume of one system is 500 ml. and the volume of another system is 100 ml., an addition of 10 ml. of gas to each of the systems will cause the pressures of those systems to change. The extent of these changes will be $\frac{10}{500}$ and $\frac{10}{100}$.

$\frac{10}{500} \times 760 = 15.2$ mm. increase in pressure or 775.2 mm. total pressure.

$\frac{10}{100} \times 760 = 76.00$ mm. increase in pressure or 836.00 mm. total pressure.

Thus it is clear that the pressure change in the 500-ml. system is less than the pressure change in the 100-ml. system when equal volumes of gas are introduced into them.

Table I shows the relation between changes of manometric readings of the thermobarometer and the corresponding changes of each of the individual respirometers. This table is based on the values obtained by using

TABLE I

MANOMETRIC READINGS FOR EQUAL QUANTITIES OF GAS INTRODUCED INTO RESPIROMETER SYSTEMS OF DIFFERENT VOLUMES

	CONTROL RESPIROMETER (THERMOBAROMETER	RESPIROMETER 1	RESPIROMETER 2	RESPIROMETER 3	RESPIROMETER 4
Volume in mm.³	90512	94686	90475	88945	99885
Change in manometric reading in mm.	30	28.68	30.00	30.53	27.18
	60	57.36	60.00	61.06	54.36
	90	86.04	90.00	91.59	81.54

the volume of the control respirometer as the numerator and the volume of the respirometer in question as the denominator.

As has been shown, the larger volume would be affected least by an addition of a unit of gas, thus: $\frac{90512}{99885} = 0.906$, showing that a change of 1 mm. of the control manometer would be analogous to a change of 0.906 of the manometer of respirometer 4. Of course, the larger the volume of gas which is introduced into the systems becomes, the more conspicuous this difference becomes, until a change of 90 mm. of the control manometer would indicate a change of only 81.54 mm. in the manometer of respirometer 4.

As the volumes of the vessels become smaller the differences between the volumes of the control and the experimental respirometers must become proportionately smaller if the ratio between the volumes is to be kept near 1. Should the control respirometer and an experimental respirometer have respective total volumes of 9 and 10 ml., the correction factor for the experimental respirometer would be $\frac{9}{10} = 0.9$, or practically the same as the correction factor obtained in the preceding paragraph where the difference in volume of the two systems is 10 ml. while in the present instance the difference is but 1 ml.

It is important to note that a complete table of the type suggested by table I cannot be constructed unless a constant volume of material is to be used. In such an instance the volume of the material and reagents should be deducted from the total volume of the systems of the experimental respirometers. The volume of the reagents contained in the thermobarometer should also be deducted from its total volume.

Suppose, during the experiment, the control respirometer (10-ml. volume) showed a manometric reading of 30 for CO_2 and 1 ml. of the reagents and respirometer no. 1 (12-ml. volume) showed a manometric reading of

80 for the CO_2 produced by 1 ml. of respiring material plus the amount contained in 1 ml. of the reagents. The usual procedure has been to subtract 30 from 80 for a reading of the CO_2 produced by the respiring material. The fallacy in this is shown by the following calculations.

The vessel constants of the systems are computed for 0° C. using the formula which has been given.

$$\frac{(10-1)\times\frac{273}{273}}{10} = 0.9 \text{ for the control respirometer and}$$

$$\frac{[12-(1+1)]\times\frac{273}{273}}{10} = 1 \text{ for respirometer no. 1.}$$

$80 - 30 = 50$ manometric graduations of respirometer no. 1.

50×1 (vessel constant of no. 1) $= 50.0$ mm.^{3} CO_2 by the usual method.

A change of 30 graduations of the control, however, would be equal to 0.9×30 graduations of no. 1, therefore:

$80 - (0.9 \times 30) = 53$ manometric graduations of no. 1

$53 \times 1 = 53.0$ mm.^{3} CO_2 by suggested procedure

or if one prefers to deal with actual volumes of gas instead of manometric graduations the conversion for the same instance will be:

$$(80 \times 1) - (30 \times 0.9) = 53.0 \text{ mm.}^3 \text{ of } CO_2.$$

The difference shows

$\frac{(53-50)}{53} \times 100 = 5.66$ per cent. too little CO_2 measured by the usual procedure.

In an experiment using a control respirometer of 92 ml. and an experimental respirometer of 112 ml., suppose the control showed a manometric reading of 30 while the manometer of experimental respirometer no. 2, which contained 10 ml. of respiring material plus 2 ml. of reagents, read 80 for the production of CO_2 at 0° C.

Then:

$$\frac{(92-2)\times\frac{273}{273}}{10} = 9, \text{ the vessel constant for the control,}$$

and

$$\frac{[112-(10+2)]\times\frac{273}{273}}{10} = 10, \text{ the vessel constant for no. 2.}$$

The conversion factor in this instance is the same as in the previous experiment for $\frac{90}{100} = 0.9$, therefore:

$0.9 \times 30 = 27$

$80 - 27 = 53$ manometric graduations of no. 2

$53 \times 10 = 530$ mm.^{3} of CO_2 by the suggested procedure

and

$80 - 30 = 50$ manometric graduations of no. 2

$50 \times 10 = 500$ mm.^{3} of CO_2 by the usual procedure.

The difference shows

$\frac{530 - 500}{530} \times 100 = 5.66$ per cent. too little CO_2 measured by the usual procedure.

In this second instance the percentage difference is the same but the amount of gas concerned is ten times as much as in the first instance. It may be repeated that the smaller the vessel constant of a system, the greater is the accuracy of manometric readings for that system. While the effect of the difference in volume between the control and experimental respirometers may be considered negligible in some studies (when the vessels are of 10 to 15 ml. capacity), in investigations where larger (100-ml.) respirometers are used the effect of this difference in volume would necessarily be considered in order to obtain the greatest possible accuracy for the experiment.

Summary

A study of the operation of Warburg respirometers has been made in connection with an investigation of the oxygen consumption and carbon

SAMPLE RECORD SHEET

	EXPERIMENTAL RESPIROMETER		THERMOBAROMETER OR CONTROL	
Dry wt. of sample in grams	10.4476			
Volume of sample and /or reagents in ml.	17.6		2.1	
Volume of system in experiment in mm.^{3}	87810 − 17600 = 70210		90512 − 2100 = 88412	
	TIME	READING	TIME	READING
Set in bath	9:55		9:55	
Seeds placed in vessel, start CO_2	10:02 (*a*)			
Stopcock closed, start O_2	10:05 (*c*)	150.5 (*b*)	10:05 (*c*)	149 (*b*)
End O_2, dump HCl	10:35 (*d*)	123 (*e*)	10:35 (*d*)	151 (*e*)
End CO_2, end of mixing	10:38 (*g*)	167 (*f*)	10:38 (*g*)	181.5 (*f*)

	EXPERIMENTAL RESPIROMETER GRADUATIONS	CONTROL CORRECTION FOR EXPERIMENTAL RESPIROMETER	CONTROL
Change, O_2	150.5 − 123 = 27.5	2	151 − 149 = 2
Corrected O_2	27.5 + 2 = 29.5		
Change, CO_2	167 − 123 = 44	$\frac{88412}{70210} \times 30.5 = 38.40$	181.5 − 151 = 30.5
Plus O_2 consumed in 3 minutes	(44 − 38.40) + 2.95		
Corrected CO_2	8.55		

CALCULATIONS

VESSEL CONSTANT (0° C.)	× MANOMETER GRADUATIONS	× TIME CORRECTION	= TOTAL GAS IN MM.³ (30 MIN.)
$\frac{87810 - 17600}{10000} = 7.021$	8.55	$\frac{30}{36} = 0.8333$	50.02 mm.³ CO_2
7.021	29.5		207.12 mm.³ O_2

$$\frac{\text{Total } CO_2}{\text{Total } O_2} = \text{R. Q. or } \frac{50.02}{207.12} = 0.2415$$

TOTAL GAS	÷ DRY WT. OF SAMPLE IN GM.	× TIME CORRECTION	= MM.³ OF GAS PER GM. DRY WT. PER HOUR
50.02	10.4476	2	= 9.58 mm.³ CO_2
207.12	10.4476	2	= 39.64 mm.³ O_2

dioxide production of acorns. It was found that the accuracy of the method could be increased by modifying the usual technique in three ways. A correction should be made for the absorption of oxygen during the interval between the final oxygen reading and the final carbon dioxide reading. Theoretically, a correction should be considered for the solubility of these gases in the reagents before and after mixing, but since reliable solubility values are frequently not available for these reagents more accurate results may be obtained by omitting a correction for solubility. Differences in volume of respirometers prevent the direct application of a control correction for carbon dioxide in the reagents. A sample set of recordings and calculations are presented.

The writer wishes to express sincere appreciation to Dr. P. J. KRAMER of the Department of Botany and to Dr. F. G. HALL of the Department of Zoology of Duke University for material assistance in the preparation of this paper.

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RELATION BETWEEN FRUIT SIZE AND FOOD SUPPLY IN THE TOMATO[1]

FELIX G. GUSTAFSON AND HELEN B. HOUGHTALING

(WITH FIVE FIGURES)

Recently GUSTAFSON and STOLDT (2) made a study of the relation between leaf area and fruit size of the tomato. In this study it was found that by increasing the leaf area per fruit, the size of the fruit produced was also increased. In their experiments the leaf area was not adjusted until after the ovary had completed its growth and the fruits had set. It has therefore seemed desirable to attempt to influence the size of the fruit while the ovary is being formed as well as during the period of its development into a fruit. In the present study we have measured the volume of the ovaries at the time of blossoming, the volume of the mature fruits, the cell size of these ovaries and mature fruits, and the diameter of the pith of the stem just below the insertion of the flower cluster; and we have also counted the number of locules in the fruits measured.

Procedure

About 700 experimental plants (John Baer) were set out in the field and 200 were left in the greenhouse, in 8-inch pots. These were all pruned to one stem and staked; and as new buds appeared in the leaf axils they were removed to keep the plant in the one-stem condition. Next to the experimental plants in the field were available, for comparison, other John Baer plants grown in the usual way with many branches and not staked. These bore heavily during the early part of the season. Beginning with the third cluster all fruits set were allowed to develop on the control plants. The experimental plants, on the other hand, were permitted to bear fruits in only one cluster per plant, and not more than eight fruits per cluster. At the time of blossoming 117 plants were selected for bearing in the third cluster, 120 for the fourth, 124 for the fifth, and 97 plants bore fruits in the sixth cluster. Many of these plants did not develop properly and the number of plants from which the fruits were harvested was somewhat smaller than the original number of plants selected at the time of blossoming. Attempts were made to select uniform plants which had unbranched flower clusters; but this was not entirely feasible for the fifth and sixth clusters.

The growing conditions were very satisfactory until about the middle of July when the temperature became quite high and remained so for several weeks. There was also very little rain during this time but the latter con-

[1] Paper from the Department of Botany and Botanical Gardens of the University of Michigan, no. 636.

dition was remedied by one thorough watering of the ground in which the plants grew. The flowers in the fifth and sixth clusters were, for the most part, produced during the hot, dry weather. The flowers and the fruits produced were, however, normal in appearance, though it is impossible to say what effect this weather condition may have had upon the size of the ovary and later upon the fruit.

As the flowers opened in the different clusters, the first five flowers in each cluster of the experimental plants were picked and the ovaries preserved. The ovaries were sectioned longitudinally and length determined. These flowers of course were picked not from the plants that were permitted to set fruits in this cluster, but from a different group of plants. In the control culture, where there were no extra plants, the first flower in a cluster was removed from 20 plants, likewise the second flower in the cluster was removed from 20 other plants, and similarly flowers were removed from the third, fourth, and fifth position in the cluster from other plants. In this way only *one* flower was removed from any one cluster. The ovaries from these flowers were sectioned and measured as in the experimental plants. When mature, the fruits were picked, and the diameter and length measured. The fruits were also cut crosswise to count the number of locules. From the first 5 fruits in each cluster, from 20 plants of each lot of the experimental plants, and from all of the fruits of 20 control plants, sections for cell-size study were removed and preserved. These sections were always taken from the same part of the fruit, namely the equatorial region, and only the ovary wall or pericarp was used. After all the fruits had been removed from these plants the stems were cut a few centimeters below and above the insertion of the fruiting cluster. The pieces of stems, properly labeled, were taken into the laboratory where hand sections were made four to five centimeters below the cluster, and the pith diameter measured with a microprojector.

The volume of the ovaries and fruits was obtained by multiplying the squared diameter by the length. This gave the volume of a cube having the dimensions of the fruits. Inasmuch as the correction factor for the volume of a sphere would be the same for all and as we were interested only in relative values we did not make this correction.[2]

For the cell size determination, hand sections stained with Delafield's hematoxylin were used. HOUGHTALING (3) has found that these cells are approximately spherical. The cells from the ovaries were measured by the aid of a micrometer eye-piece and high magnification. The cells from the mature fruits were projected, traced on paper and the longest diameter and the diameter at right angles to it were measured. Ten cells from each fruit

[2] If the fruit is considered to be an oblate spheroid the approximate volume would be obtained by multiplying our values by $\pi/8$.

were measured. This made 50 cells from each cluster, and as 20 plants were used, 1000 cells were measured for each level on the experimental plants. That is, 1000 cells were measured from the fruits growing in the third cluster, 1000 from the fourth, etc.; in all, 4000 cells were measured from the fruits of the experimental plants. The average number of fruits per cluster of the control plants was approximately 2.3; the total number of cells measured per cluster of the control plants therefore was about 460, or a total of 1840 cells from the control plants.

Results

The first question to be answered was: what is the influence of increase in size of the plant upon the size of the ovary? As mentioned in the section

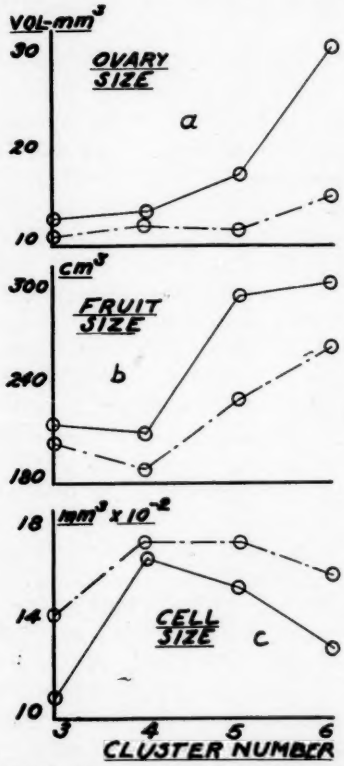

FIG. 1. Solid lines denote the experimental plants, interrupted lines the control plants. Graph *a*, average volume of the ovaries of the first five flowers from the different clusters; graph *b*, average volume of mature fruits; graph *c*, average volume of the cells from mature fruits from different clusters.

on procedure, the experimental plants produced fruits only in the third, fourth, fifth, or sixth cluster, *i.e.*, in one cluster per plant. It is obvious that the plants that bore fruits in the fifth cluster were much larger, and presumably had stored more food when the flowers were being produced, than those plants that bore fruits in the third cluster. Figure 1*a* shows that in the experimental plants the older and larger plants, *i.e.*, those plants that bore fruits in the fifth and sixth clusters, produced ovaries that were larger than those produced by plants that were younger and smaller. There was, however, no constant relation between plant size and ovary size. In the control plants, which were supplying food to growing fruits at the same time that the ovaries were forming, the ovary size was only very slightly larger in successive clusters. Evidently the nutritional level of the plant at the time the flower bud develops greatly influences the size of the ovary formed in the bud.

Figure 1*b* gives some very interesting information concerning the size of the fruits developed under the different nutritional levels. The larger ovaries in the fifth and sixth clusters of the experimental plants did not grow into correspondingly larger fruits. They were larger than those produced from smaller ovaries but not proportionately to the difference in the original ovaries. This is especially true of the sixth cluster, where the volume of the ovaries of the control plants was only 57 per cent. of the volume of the ovaries of the experimental plants, while the mature fruits were 87 per cent. as large as the corresponding fruits in the experimental plants. The volume of the ovaries of the control plants in cluster three was 81 per cent. of that of the ovaries of the experimental plants, while the mature fruits were 95 per cent. as large. The fruits from the control plants are thus seen to have gained on the fruits from the experimental plants during their development. Obviously this was brought about either by the production of more new cells in the control fruits than in the experimental fruits during the period between flowering and fruit maturation, or the cells were larger in the control fruits. It is possible that both of these conditions occurred together. HOUGHTALING (3) has shown, however, that little or no cell division occurs in the tomato after the setting of the fruit, and this has been verified in the present investigation. That leaves the matter of difference in cell size. Figure 1*c* shows the size of the cells in the fruits from the different clusters in the two groups of plants. The control fruits have larger cells throughout than the corresponding experimental fruits. Is this difference sufficient to account for the greater size in the fruits of the control plants? Table I shows the percentage relationship between the volume of the ovaries, fruits, and cells of the control and experimental plants. In cluster three the fruits from the controls gained 14 per cent. on the experimental fruits, while the cell size of the former was 32 per cent. greater than

TABLE I

PERCENTAGE OF THE VOLUME OF THE OVARIES, FRUITS, AND CELLS OF THE CONTROL IN TERMS OF THE EXPERIMENTAL PLANTS

CLUSTER NUMBER	OVARIES*	FRUITS*	CELLS*
	%	%	%
3	81	95	132
4	94	91	105
5	68	78	114
6	57	87	124

* Values obtained by dividing those of the control plants by the corresponding values for the experimental plants.

that of the latter. This is a rather large discrepancy. In the remaining clusters the difference is not so great.

If we grant that the greater increase in the control fruits is entirely attributable to the larger cells, there is still left the problem of accounting for these larger cells. GUSTAFSON and STOLDT (2) found that when the leaf area per fruit was increased, the volume of the fruits increased as much as 40 per cent. In these experiments we have increased the size of the ovaries as much as 73 per cent. (in cluster 6) by preventing the plants from setting fruits, yet fruits from these ovaries were only 14 per cent. larger than the control fruits grown from the much smaller ovaries. There seems to be a limit to the size of a fruit that a plant can produce; beyond that it cannot grow.

The large fruits were very irregular in shape, while the smaller ones were regular and symmetrical. To determine the cause of this irregularity we began to cut the fruits crosswise and noticed that the larger fruits were composed of more carpels, *i.e.*, had more locules, and that the carpels were very crowded and, due to the crowding, often misshapen. This find led us to count the number of locules per fruit. When all of the fruits studied were analyzed it was found that there was a positive correlation of 0.8421 ± 0.0431 between the number of locules and the fruit size.

A comparison between the size of the fruits produced by the plants pruned to one stem and those grown on plants with a number of stems, and bearing heavily all summer long, is very interesting. From the latter group 100 ripe fruits were collected at the time the fruits were collected from the sixth cluster of the experimental and control plants. While the position of these fruits on the stem was not determined, they were probably from the fifth or sixth cluster. A comparison between the size of these fruits and that of the fruits from the sixth cluster of the control and experimental plants will therefore not be far wrong. These values are: for the experimental fruits, average volume 303 ml., for the control 265 ml. and for the

fruits from the normally grown plants only 154 ml. The fruits from the experimental plants were nearly twice as large as those from the ordinary plants. This again brings out the fact that one can increase the size of a fruit, though evidently not indefinitely. Another comparison which can be made is that between the cell sizes of these classes of fruits. The average size of cells from the fruits from the sixth cluster of the experimental plants was 13.161 mm.^{3}, from the control fruits, 16.255 mm.^{3}, and from the normally grown fruits, 10.304 mm.^{3}

We were also interested in the relation between the size of the ovary and its position in the fruiting cluster. It has been generally recognized that the fruits first produced in a cluster are the largest (GUSTAFSON, 1). Figure 2 shows that the same is also true for the size of the ovary in control

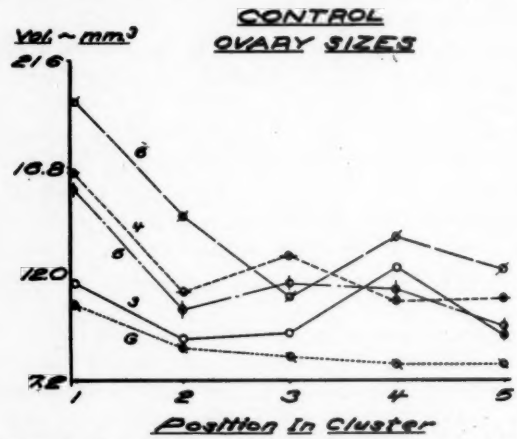

FIG. 2. Volume of the ovaries in the different positions in a cluster. The four clusters studied in the controls are represented. Curve G, volumes of the ovaries from the fifth and sixth clusters of the greenhouse grown plants.

plants. This is not always true, however, when the plants have a high nutritional level during the development of the flower buds (fig. 3). In cluster three the first and the fifth ovaries were of the same size, and in the fifth cluster the fifth ovary was much larger than the first. In the sixth cluster, which flowered during the hot dry weather, there was a very large difference in size between the first and the second ovary. The experimental plants produced more flowers in the sixth cluster than they had in the other clusters. Whether the production of this large number of flowers was a sufficient drain upon the food supply entering the fruiting branch to decrease so sharply the size of the ovaries, or whether the hot and dry weather was the cause, we do not know. It should be noted that even though the fifth ovary

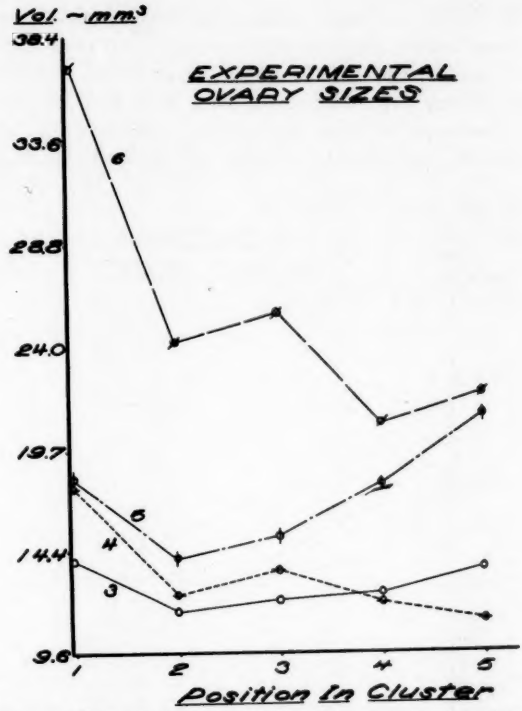

FIG. 3. Same as figure 2, but representing the experimental plants. Unlike the ovaries from the control plants there is not always a decrease here in size in successive ovaries in a cluster.

in cluster six is much smaller than the first one it still is larger than the first ovary in the control plant for the same cluster.

As mentioned above, it is usually stated that there is a decrease in the size of the fruit with the order of development in a cluster. This is borne out by figure 4. The results in the experimental fruits (fig. 5) are somewhat variable. Cluster 3 is very much like the corresponding one in the control plants, and there is no reason why it should be different, as it is the first bearing cluster in both groups. Clusters 4 and 5 are very different in the two groups. In cluster 5 of the experimental plants the sixth fruit is 68 per cent. larger than the fruit in position one; in the corresponding cluster in the control the fifth fruit (which is the last one) is only 52 per cent. as large as the fruit in position one. In the sixth cluster of the experimental plants the fruits, like the ovaries, are decidedly peculiar. The sixth fruit is smaller than the first, but not so much smaller as was the ovary.

The results for the sixth cluster in the experimental plants are based on a smaller number of plants than any of the others; and some of the fruits last produced may not have been fully grown, as they had to be picked before they were completely ripe. This, together with the fact that the later ovaries were much smaller than the first one, may account for the smaller size of the fruits later produced in cluster 6 of the experimental plants.

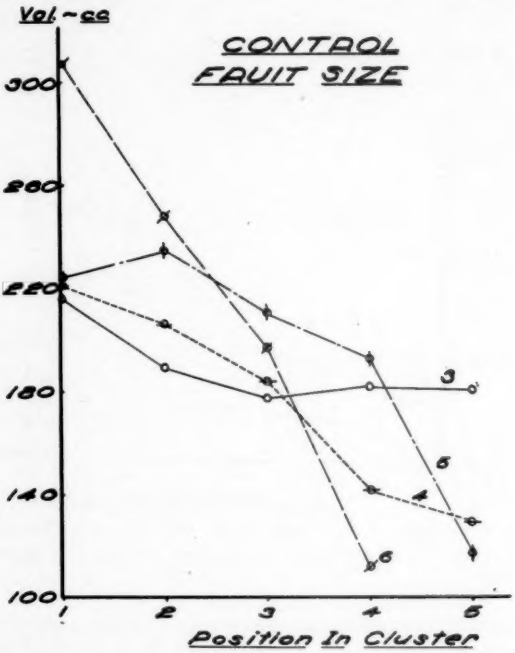

FIG. 4. Volume of the fruits in the different positions in a cluster. The four clusters studied in the control are represented.

SINNOTT (4) from his work on the bean and on *Acer saccharum* came to the conclusion that there is no correlation between the size of the body of a plant and the organs produced by it, such as leaves and fruits, but that there is a correlation between the size of the growing point and the body produced by it. It was impractical to measure the size of the growing point so he conceived the idea of measuring the diameter of the pith of the stem near the point where the organ was located. He considered that, as the pith was laid down by the growing point, it bore a certain relation to the size of the growing point. He measured the leaves of *A. saccharum* and the diameter of the pith in the internode below the attachment of a leaf and found a

correlation between the size of the pith and the size of the leaves coming from the stem nearby.

Having the fruit volume already, we thought it worth while to determine whether in the tomato there was also a correlation between pith size and size of the organ (fruit) produced by the stem. We therefore measured the diameter of the pith of 20 stems for each group of experimental plants; and the pith of 20 control plants was measured below each cluster of fruits. The greenhouse plants were also used. The pith diameter was measured from hand sections from the internode below the fruits. There was found to be

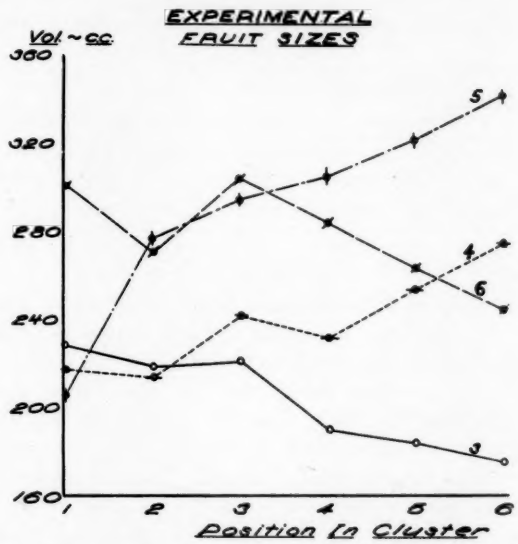

FIG. 5. Same as figure 4, but representing the experimental plants.

no correlation for the individual groups, but when the three groups were brought together there was a positive correlation of 0.6230 ± 0.0505 between pith diameter and fruit volume.

Discussion

These experiments show beyond a doubt that the size of the fruits can be increased considerably by increasing the size of the vegetative structure before the time at which the flowers are produced, but it is equally obvious that at least in the John Baer tomato the size cannot be increased indefinitely. If the ovaries are increased beyond the normal, then the cells do not enlarge as much as they do in fruits that are developing from smaller ovaries.

How are we to explain this? SINNOTT would say that the growing point was larger at the time the fruit buds were initiated and the larger meristem produced larger ovaries. Unfortunately we did not measure the size of the ovaries from the same plants in which we measured the pith diameter; and we have the pith diameters for only 20 plants for each cluster in the experimental plants and 20 plants all told for the control plants. The data for the pith diameters and the size of the fruits produced in the clusters just above are given in table II. The table shows that in the control, in which

TABLE II

AVERAGE VOLUME OF THE FRUITS PRODUCED IN A CLUSTER AND THE PITH DIAMETER OF THE INTERNODE JUST BELOW

No. of cluster	Experimental fruits		Controls	
	Diameter of pith	Size of fruit	Diameter of pith	Size of fruit
	mm.	*ml.*	*mm.*	*ml.*
3	7.0	226.2	7.2	214.2
4	6.9	216.5	6.2	213.9
5	6.4	283.9	5.1	244.7
6	7.3	289.5	5.3	328.4

the diameter of the pith below successive clusters of the same 20 plants was measured, the pith diameter decreased upward so that the diameter below the third cluster was much larger than that below the sixth; yet when we refer to figure 1*a* we note that the ovaries from the sixth cluster were somewhat larger than from the third. The size of the mature fruits was much larger from the sixth cluster, with the smaller pith, than from the third cluster, below which the pith was the largest. The correlation between pith size and fruit size for the control is -0.0164 ± 0.1103. There is less variation in the size of the pith from the different levels in the experimental plants. Again referring to figure 1*a* we note the very much larger ovaries produced by the experimental plants in the sixth cluster than in the third, though the pith of the mature plants was only slightly larger below the sixth cluster. The correlation between pith size and fruit size in the experimental plants was only 0.3248 ± 0.1023. In both the control and experimental plants the correlation between pith diameter of the stem and the average size of the fruits produced at that point of the stem is not significant.

Another matter to be discussed is that of the cell size. Why do the cells grow larger in the smaller ovaries of the control fruits, so that the resulting fruits are nearer to the size of the experimental fruits than one would expect them to be? There is here a leveling off as far as the size is concerned; the originally larger ovaries grow less than the smaller ovaries. In

an earlier paper GUSTAFSON (1) has suggested that the size of the fruit may be determined by the conduction of food and water into the enlarging fruit. The vascular system within the fruit of the John Baer tomato is very poorly developed and if the demand upon it becomes too great it is likely that its capacity for conduction may not be sufficient for the need of the fruit and the results will be small cells if there are many of them in a fruit. It seems to us, therefore, that the poor distribution of food materials in the fruit tends to keep the fruit down to a maximum size beyond which they cannot grow. If the ovaries contain many cells, these cells do not grow as large in the maturing fruits as they do in fruits having fewer cells in the ovary stage.

Summary

1. In these experiments 4 lots of John Baer tomato plants have been used: (a) plants grown in the greenhouse; (b) plants grown in the field not pruned; (c) field plants pruned to one stem, some of which were allowed to mature all the fruits that were set, beginning with the third cluster; and (d) other plants pruned as in (c) which were allowed to bear in only one cluster per plant.

2. The field plants that were pruned to one stem produced fruits that were nearly twice as large as the fruits produced by the unpruned plants.

3. By further reducing the number of fruits produced to one cluster per plant both the ovaries and the mature fruits were greatly increased in size, but the fruits were not increased proportionately to the larger ovaries. This was caused by the fact that the cells in the fruits produced from the larger ovaries were not so large as in the fruits produced from the smaller ovaries on the control plants. One can then state that *by increasing the leaf area per fruit, the individual fruits grow much larger but there is a limit to the size of the fruit produced, dependent upon the variety.*

4. In the controls, both the ovaries and the mature fruits that were produced later in a cluster were smaller than those produced first. This was usually not true in the experimental plants.

5. There was no significant correlation between pith size of the stem and the size of the mature fruits produced at the same level on the stem, and we are of the opinion that there is a relation between the size of the plant and the fruit produced by it, *i.e.*, a large well-nourished plant bears larger fruits than does a small plant.

6. There was a significant correlation between the fruit size and the number of carpels composing it.

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ORGANIC ACID METABOLISM OF THE BUCKWHEAT PLANT[1]

GEORGE W. PUCHER, ALFRED J. WAKEMAN, AND
HUBERT BRADFORD VICKERY

Introduction

Much of the recorded information concerning the metabolism of the organic acids in plant tissues has been obtained from measurements of diurnal changes either in the titratable acidity or in the hydrogen-ion activity of succulent plants. Such data yield little definite information with regard to the behavior of the individual acids present in the tissues. The calculation of the results of the titration of the organic acid in terms of grams or milliequivalents of any specific acid is usually based on the assumption that the predominating acid is the only one involved in the changes that may be observed. Although this is to some extent justifiable in many cases, the evidence is seldom satisfactory. BENNET-CLARK (**1**), in his careful review of the whole subject, points out that information on the intermediary metabolism of organic acids in plants is so fragmentary that "general pronouncements on the significance of the plant acids are at present probably of little value . . . and our knowledge of plant acid metabolism is therefore best summarized by including all the reactions which have been shown to occur in living tissues since it is usually impossible to show that such reactions do not occur in any given tissue."

Although the organic acid metabolism of plants other than succulents has been studied less extensively than that of the succulent plants, it is known that diurnal variations in pH occur in many species. Such changes have been interpreted by CLEVENGER (**2**), in the case of the cowpea, to signify an accumulation of organic acids during the night and utilization during the day. The changes he observed, however, are not nearly as pronounced as those often found in the Crassulaceae, and both GUSTAFSON (**3**) and HURD (**4**) have reported that the diurnal fluctuations in the pH of nonsucculents are not always associated with changes in the titratable acidity. SMALL (**16**) states that the variation in pH brought about by differences in the intensity of illumination of the plants is affected not only by the total concentration of acid but also by other factors connected with the metabolism of the living leaf, possibly the type of buffer system present, or the nature of the individual acids.

[1] The plants described in the present communication were grown at the New Jersey Agricultural Experiment Station. It is a pleasure to thank Dr. SHIVE and Dr. ROBBINS for the supply of seed, for their help and cooperation, and for the hospitality of the laboratory to one of us. A part of the expense of this investigation was borne by the Carnegie Institution of Washington.

The present study was undertaken to obtain preliminary data on the variations in the organic acids of a nonsucculent plant that shows diurnal periodicity in pH. Buckwheat (*Fagopyrum esculentum* Moench) was chosen since INGALLS and SHIVE (**5**) have shown that the pH of the sap expressed from both leaves and stems increases during a period of maximum illumination and decreases during the night.

Experimentation

The seeds were germinated between moist filter papers and were transferred to a germinating net as described by SHIVE (**13**). On April 4, 1937, when the seedlings were about 5 cm. tall, 72 plants were selected for uniformity of size and vigor, and were transferred, in groups of six, to 12 two-quart jars filled with a nutrient solution of the following composition: KH_2PO_4 0.00225 M, $Ca(NO_3)_2$ 0.0045 M, and $MgSO_4$ 0.00225 M. The nutrient solution also contained 0.5 to 1 part per million of iron and 0.25 parts per million each of boron and of manganese. The solution cultures were aërated continuously, and SHIVE's continuous-flow method of supplying fresh nutrient at the rate of about 500 to 700 ml. per day was employed. On April 28, the plants had attained a height of 55 to 60 cm. and were beginning to form flower buds. April 30, 26 days after transplantation, promised to be a bright and cloudless day so that maximal differences in light intensity during the sampling period seemed assured. Three cultures containing a total of 18 plants were therefore harvested at the start, and at each of three successive intervals during a 22-hour period. The plants were divided into leaf and stem tissue which were separately weighed and dried in a ventilated oven at 80° C. A 10-gm. aliquot of each fresh stem sample was frozen and the pH of the expressed sap was determined with a quinhydrone electrode. To conserve material for subsequent chemical analyses, pH determinations on the leaf tissue were omitted, since INGALLS and SHIVE have shown that the pH changes in the blade are similar in magnitude to those in the stem.

The four samples of leaf and stem tissue were obtained, one at 6:20 A.M., E.S.T., a second at 1:45 P.M., a third at 7:00 P.M., and a final one at 4:00 A.M. the following morning. The organic acid content of the dried samples was subsequently determined by specific methods (**6, 7, 8, 9**).

ORGANIC ACIDS OF BUCKWHEAT

Although extensive studies on the nutrient requirements of the buckwheat plant have been carried out by SHIVE (**14, 15**), there are, so far as we are aware, no data available on the organic acid composition of this plant. Table I shows the organic acid content of the initial sample of tissue in percentage of the dry weight. The chief acid of this plant is oxalic, the con-

TABLE I

ORGANIC ACID COMPOSITION OF BUCKWHEAT PLANT*

ACIDS DETERMINED	LEAF†	STEM†
	%	%
Oxalic acid	11.1	3.95
Malic acid	1.51	1.96
Citric acid	0.82	0.46
Nitrate N	0.37	2.07
Ash	13.27	17.48

* The average fresh weight of a single plant was 11.00 gm., of which 3.04 gm. were leaf, 7.19 gm. stem, and 0.765 gm. root. The average dry weight was 0.962 gm. distributed as 0.406 gm. leaf, 0.481 gm. stem, and 0.0753 gm. root.

† Data are given in percentage of the dry weight.

centration in the leaf being about three times as high as that in the stem. Malic and citric acid are also present in appreciable quantities, the former being slightly higher in the stem than in leaf tissue. Over 90 per cent. of the total organic acidity in the leaf is present as oxalic, malic, and citric acids, while in the stem only about 72 per cent. can be accounted for in terms of these three.

The stems of the plants contained a concentration of nitrate nitrogen more than five times greater than that of the leaf tissue. The quantity found is, in fact, the equivalent of nearly 15 per cent. of the dry weight, when expressed as potassium nitrate, and it is evident that a large part of the cations of the stem must be combined with nitrate ions. Accordingly a large proportion of the organic acids must be free or in the form of monobasic ions. This may account for the observation that buckwheat stem tissue, in spite of its greater ash and smaller organic acid content, is more acid in reaction than the leaf tissue.

Results

The behavior of the organic acids of buckwheat during the day may be most simply presented in terms of the changes in actual amount of each acid during each of the three successive time periods. These were selected to give first, an interval during which the illumination increased to a maximum and the reaction of the tissues, according to INGALLS and SHIVE, might be expected to become less acid; second, a period during which the light intensity diminished and the reaction of the tissues might be expected to become more acid; and last, a period of darkness when little or no change in pH occurs.

The changes are recorded in the tables as grams or milliequivalents, and also in percentage of the total amount of each substance present at the beginning of each successive period. Table II shows the changes in water, organic solids, and ash; table III shows the changes in organic acids.

TABLE II

CHANGES IN WATER, ORGANIC SOLIDS, AND ASH OF BUCKWHEAT PLANTS DURING THREE SUCCESSIVE TIME PERIODS IN ONE DAY

	LEAF*		STEM*		LEAF + STEM*	
	Period I, 6:20 A.M.–1:45 P.M.: increasing illumination and increase of pH of sap (4.20–4.40)					
	gm.	%	*gm.*	%	*gm.*	%
Water	+0.28	+10.63	+0.133	+ 1.98	+0.42	+ 4.50
Organic solids	+0.147	+41.7	+0.070	+17.6	+0.217	+29.0
Ash	+0.018	+33.4	+0.007	+ 8.33	+0.025	+18.1
	Period II, 1:45 P.M.–7:00 P.M.: decreasing illumination and decrease in pH of sap (4.40–4.24)					
Water	+0.197	+ 6.75	+0.914	+13.7	+1.11	+11.4
Organic solids	+0.025	+ 5.01	+0.038	+ 8.14	+0.064	+ 6.62
Ash	−0.002	− 2.78	−0.002	− 2.20	−0.004	− 2.45
	Period III, 7:00 P.M.–4:00 A.M.: complete darkness; no change in pH of sap (4.24–4.24)					
Water	+0.15	+ 4.83	+0.49	+ 6.32	+0.65	+ 5.98
Organic solids	−0.046	− 8.78	+0.113	+22.4	+0.06	+ 5.83
Ash	+0.004	+ 5.72	+0.015	+16.9	+0.019	+11.95

* The figures give the changes in grams per single plant in each period, and changes as percentage of the initial value in each period. The total amount of any constituent at the start of each period may be obtained by dividing the recorded quantitative change by the percentage change and multiplying by 100.

The first period is characterized by an increase in organic acids and, accordingly, the decrease in the acidity of the tissue extract cannot be interpreted as evidence for organic acid utilization. The observed increase of 0.2 unit in pH must have some other explanation such, for example, as an increase in absorption of inorganic cations, or the conversion of nitrate to ammonia. The increase of organic acid seems to be correlated with the photosynthetic processes in the plant since, from table II, it is apparent that the largest increase of organic solids, an approximate measure of photosynthetic activity, occurred during this period.

In the whole plant, 73 per cent. of the increase of total acids is due to oxalic acid and 27 per cent. to malic, citric, and unknown acids. Although the absolute amounts of acids formed, other than oxalic, are small, the percentage increases of these acids are large. The data also indicate that the individual acids are transported from leaf to stem tissue at markedly different rates. Thus malic acid increased 42 per cent. in the stem tissue and only 32 per cent. in the blade, while the unknown acids decreased in the

TABLE III

CHANGES IN THE ORGANIC ACIDS OF BUCKWHEAT PLANT DURING THREE SUCCESSIVE TIME PERIODS IN ONE DAY

	LEAF*		STEM*		LEAF + STEM*	
	Period I, 6:20 A.M.–1:45 P.M.: increasing illumination and increase of pH of sap (4.20–4.40)					
	meq.	%	*meq.*	%	*meq.*	%
Oxalic acid	+0.29	+28.7	+0.080	+19.3	+0.37	+25.9
Malic acid	+0.029	+32.2	+0.059	+41.9	+0.088	+38.1
Citric acid	+0.017	+34.0	+0.012	+34.3	+0.029	+33.3
Total gain of determined acids	+0.336	+29.2	+0.151	+25.6	+0.487	+27.9
Total organic acid	+0.38	+30.2	+0.13	+15.8	+0.51	+24.4
Unknown acids	+0.04	+36.4	−0.02	− 8.70	+0.02	+ 5.88
Nitrate N	+0.015	+14.1	+0.074	+10.4	+0.089	+10.9
	Period II, 1:45 P.M.–7:00 P.M.: decreasing illumination and decrease in pH of sap (4.40–4.24)					
	meq.	%	*meq.*	%	*meq.*	%
Oxalic acid	+0.04	+ 3.08	+0.017	+ 3.43	+0.05	+ 2.78
Malic acid	+0.002	+ 1.68	−0.030	−15.0	−0.028	− 8.78
Citric acid	+0.006	+ 8.70	−0.001	− 2.13	+0.005	+ 4.31
Total gain of determined acids	+0.048	+ 3.23	−0.014	− 1.89	+0.027	+ 1.20
Total organic acid	+0.13	+ 7.75	+0.055	+ 5.76	+0.18	+ 6.92
Unknown acids	+0.08	+53.3	+0.06	+28.6	+0.15	+41.7
Nitrate N	−0.020	−16.4	+0.042	+ 5.35	+0.022	+ 2.42
	Period III, 7:00 P.M.–4:00 A.M.: complete darkness; no change in pH of sap (4.24–4.24)					
	meq.	%	*meq.*	%	*meq.*	%
Oxalic acid	+0.14	+10.5	+0.058	+11.3	+0.20	+10.8
Malic acid	+0.019	+15.7	+0.097	+57.1	+0.116	+39.9
Citric acid	+0.016	+21.3	+0.013	+28.3	+0.029	+24.0
Total gain of determined acids	+0.175	+11.4	+0.168	+23.1	+0.345	+15.3
Total organic acid	+0.04	+22.6	+0.11	+10.9	+0.15	+ 5.40
Unknown acids	−0.13	−56.5	−0.06	−21.4	−0.19	−37.3
Nitrate N	−0.010	− 9.90	+0.085	+10.3	+0.071	+ 7.64

* The figures give the changes in grams per single plant in each period, and changes as percentage of the initial value in each period. The total amount of any constituent at the start of each period may be obtained by dividing the recorded quantitative change by the percentage change and multiplying by 100.

stem and increased 36 per cent. in the blade. These observations clearly show that data for the total organic acids alone cannot be used to interpret the metabolic changes in the individual acids. This has also been pointed out in the case of the organic acids of the rhubarb leaf (**12**).

In the second period, the increase of total organic acids in the leaf tissue was only one-third of that during the first period. Synthesis of malic, citric, and oxalic acid almost ceased, and 83 per cent. of the increase in total organic acids in the leaf and stem was due to the production of acids of

the unknown group. This appears to be correlated with the fact that the plants received a diminishing amount of light and showed a corresponding diminution in photosynthetic activity. The definite loss of malic acid from the stem in this period shows that utilization of malic acid may occur when photosynthetic activity in the leaves is diminished. If the malic acid that disappeared had been transported from stem to leaf, there would have been a far more extensive increase in the leaf than was actually observed.

In spite of the utilization of malic acid in the stem, and the small increase in total acids, the tissue sap became more acid, a further indication that tissue pH is not a satisfactory index of the organic acid metabolism. Table II shows that no inorganic salt absorption occurred, and it therefore seems probable that the decrease in pH is associated with the establishment of a new equilibrium between the cations and the organic acids. The utilization of a part of the nitrate in the leaf suggests that some such rearrangement must have occurred.

The behavior of the organic acids during the period of darkness is particularly interesting. Table III shows that, although the total quantity of organic acids produced during the night by the leaf and stem was only 0.15 milliequivalent, the combined increase in oxalic, malic, and citric acid was 0.345 milliequivalent. This increase is clearly attributable to a transformation of acids of the unknown group, formed during the second period, into one or more of these three substances. In the second period, 0.15 milliequivalent of unknown acids were formed, in the third this fraction decreased by 0.19 milliequivalent. The agreement is well within the experimental error of these determinations. During the night, two distinct types of reaction appear to take place: a small quantity of organic acids is produced, and the unknown acids formed during the second period are converted into one or more of the known acids. The latter process recalls the observation that malic acid is converted into citric acid during culture of tobacco leaves in water in darkness (**11**). Table II shows that the buckwheat plant gains an appreciable quantity of ash during the night. It seems probable that a balance between cations and anions is thereby maintained so that no appreciable change in tissue pH occurs.

Discussion

It is obvious that, in the buckwheat plant, diurnal fluctuation in pH cannot safely be used alone as a criterion of the changes in the organic acid composition. It would seem that only a complete study of the variations in inorganic cations and anions, as well as of the organic bases and of the individual organic acids, would yield the information necessary to explain satisfactorily the observed fluctuations in reaction of the tissue juices. No such comprehensive set of data has, to our knowledge, ever been assembled

for a plant tissue. In a recent paper (**10**), we have shown that a definite correlation exists, in the case of samples of tobacco leaf for which relatively complete ash analyses were available, between the excess of positive ions in the ash and the quantities of organic acids present in the tissue. The organic acids obviously play a major rôle in the physico-chemical relationships upon which the hydrogen-ion activity and buffer capacity of the cell fluids depend. The present results with buckwheat clearly indicate that these relationships are far from simple, and hold out little promise that they can be accounted for from such limited data as observations of the pH or of the titratable acidity.

Summary

The buckwheat plant contains oxalic acid as the chief organic acid of both leaf and stem. Malic acid is present in relatively smaller amounts and citric acid is present in minor proportions. These three acids make up about 90 per cent. of the organic acids of the leaf and about 72 per cent. of the organic acids of the stem. The leaves of one plant contain about 1.5 times as much total acid as the stem.

During the period of maximal illumination early in the day, photosynthesis is most active and the organic acids increase. Nevertheless there is a decrease in hydrogen-ion activity of the sap of the stem. In the later part of the day, malic acid is utilized and there is an increase in the group of unknown organic acids. The net change in total organic acids is a small increase, but the hydrogen-ion activity of the sap of the stem increases to approximately the early morning level. Photosynthesis is much less active in this period, and the assimilation of inorganic ions from the culture solution is also depressed.

During the night, there is a small increase in total organic acids and a marked interconversion of acids of the unknown group into one or more of the known acids takes place. There is no change in hydrogen-ion activity in the sap of the stem.

These observations show that interpretations of the changes in the hydrogen-ion activity of the sap of the plant in terms of an increase or decrease in organic acids cannot be made in the absence of specific information regarding these substances. It is obvious that such factors as absorption of inorganic ions by the roots, and the distribution of the inorganic and organic basic constituents within the tissues, as well as photosynthesis, play an important rôle in the changes in acidity that occur.

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DETERMINATION OF AMMONIA AND AMIDE NITROGEN IN CONNECTION WITH THE CHLORATE METHOD FOR NITROGEN IN PLANT TISSUES[1]

E. M. EMMERT

Introduction

In previous work with the chlorate method (**1, 2**) for determining nitrogen in plant tissues, materials were used in which the amounts of ammonia and amide nitrogen were either absent or negligible in relation to the total nitrogen present.

In working with samples containing significant amounts of ammonia compounds or amide nitrogen, it is necessary to accompany the determination with a Nessler test for ammonia. The chlorate oxidation changed all nitrogen in the plants analyzed to nitrate nitrogen with the exception of nitrogen already in the ammonia form or nitrogen in compounds which readily hydrolize to ammonia such as amides.

In some studies of nitrogen in plants this may be a decided advantage since this divides the nitrogen into two important groups:

1. Nitrates, alkaloids, proteins, and amino acids the nitrogen in which is oxidized to nitric acid and determined by phenoldisulphonic acid, and

2. Ammonia, amides, and other readily hydrolyzable nitrogen compounds the nitrogen in which is converted to ammonia and determined by the Nessler test. These are the usual compounds to be expected in plants. Of course small amounts of the rarer nitrogen compounds may be present, but it is pretty certain in the chlorate oxidation that all nitrogen in plants is either oxidized to nitric acid or caught as ammonium sulphate. The same is probably true for all nitrogen compounds in general. It will be shown later that the N in pyridine which is not determined by the regular Kjeldahl procedure was determined by the chlorate method.

In the soluble nitrogen test (**1**) on plant extracts from the more mature conducting tissue of lower petioles of rapidly growing long-day plants, most of the nitrogen is in the form of amino acids or nitrates and for practical use to determine nitrogen deficiencies the Nessler test may be omitted, but for detailed work it should be included. MURNEEK (**3**) shows that short-day plants contain considerable amide and NH_3 nitrogen. Inclusion of the Nessler test enables a more detailed study to be made, since, after treatment with H_2SO_4 and $NaClO_3$, the phenoldisulphonic test shows all active nitrogen (amino acids and nitrates) present which is in a form for immediate use in the anabolic processes, while the Nessler test shows the reserve nitrogen

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

(amides, especially that in asparagine and other compounds readily hydrolyzed to ammonia). A third group would be free ammonia found by making a direct test on the original extract, but this form is not usually found except in very small amounts in normal plant extracts.

Procedures

AMMONIA AND AMIDE NITROGEN.—Ammonia nitrogen is determined in an aliquot of the same solution as is used in determining nitrate, alkaloid, protein and amino nitrogen in the chlorate oxidation (**1, 2**).[2] If a large amount of amide nitrogen is suspected the solution should be refluxed considerably longer (about one-half hour longer) than is directed in the regular chlorate procedure (3 to 5 minutes) to insure complete hydrolysis of amides although as a rule this is unnecessary in plant samples. This may be tested by Nesslerizing with and without extra refluxing.

Put an aliquot such that between 0.05 and 0.30 mg. of NH_3 are present into a 200-ml. Erlenmeyer flask. Usually 1 to 5 ml. are sufficient. Add almost 20 ml. of water and a small piece of litmus paper. Add strong ammonia-free NaOH until the paper just turns blue. Now add normal hydrochloric acid until the paper just turns red. Remove the paper and cool the solution to about room temperature under a tap. Make the volume to about 90 ml. with water. Add 3 ml. of Rochelle salt reagent (**4**) and 3 ml.

[2] Put 100 to 300 mg. of dry tissue, or 500 to 1500 mg. of green tissue, into a 200-ml. Erlenmeyer flask. There should be at least 0.5 mg. of nitrogen in the sample used. Add 2 ml. of 50 per cent. sodium chlorate solution for each 100 mg. of dry sample, or each 500 mg. of green sample. Mix well with the sample and allow to soak a few seconds. Add 25 ml. of 52 per cent. by volume sulphuric acid and attach the flask to a water-cooled reflux condenser. Arrange the connections to the condenser in such a manner that the flask can be shaken readily. Heat rather slowly at first. When the reaction begins to become active, shake to wash down particles from the sides. Do not allow blackening of particles on the sides of the flask if it can be helped. However, if the particles of samples have been soaked with a 50 per cent. chlorate solution some blackening does no harm. Heat until the sample is completely gone (2 to 3 minutes). Stop the heating while there is still a very faint yellow chlorine color. Flush out the condenser with two successive 5-ml. portions of 52 per cent. H_2SO_4, and cool the flask in a cold water bath. After detaching, cool to room temperature.

Make the cooled solution up to a convenient volume. If the quantity of nitrogen present is small, the volume should be as small as possible. Immediately put exactly 1 or 2 ml. (use an accurately calibrated pipette) of the solution into a 25-ml. test-tube and add 3 or 6 ml. of phenoldisulphonic acid, mix well, and allow to stand about one-half minute. (If a very reddish solution forms here, the heating was not continued long enough to decompose excess chloric acid. The solution should remain almost colorless.) Wash into a 200-ml. Erlenmeyer with about 10 or 20 ml. of water and make alkaline with 40 per cent. NaOH solution, adding it until the maximum yellow color is produced and a small excess of alkali is present. Make to a volume which brings the yellow color near to the standard. After thorough mixing, the clear solution is compared with a standard (1 ml. = 0.0025 mg. N) in a colorimeter.

of Nessler reagent (**4**). Make to exactly 100 ml. and compare with a standard (**4**) containing 0.001 or 0.002 mg. of NH_3 per ml. The solution should be read within one-half hour.

TOTAL NITROGEN.—Convert the NH_3 found to N as NH_3 by multiplying by 14/17. Add this N to the nitrate nitrogen found by the use of phenoldisulphonic acid (**1, 2**). This sum gives the total nitrogen in the sample.

TOTAL SOLUBLE NITROGEN IN PLANT EXTRACTS.—Oxidize 0.5 to 1.0 ml. of the plant extract by chlorate and fuming sulphuric acid as directed in the rapid soluble nitrogen test (**1**). After the fumes have been blown out by means of a bent glass tube until the solution is no longer yellow, wash the solution into an Erlenmeyer flask with 20 ml. of water and reflux at least one-half hour if much amide N is thought to be present. This is not necessary in many plant extracts where only small amounts of amides are present. A test with and without refluxing will determine this. Cool and treat the solution exactly as in the above directions for ammonia nitrogen.

If considerable plant extract is available the nitrogen may be determined as for total nitrogen in solids. Place 5 to 20 ml. of extract (depending on the N present) in a 200-ml. Erlenmeyer flask with 1 gm. of sodium chlorate. Attach to a reflux condenser, pour a volume of concentrated sulphuric acid (95 per cent.) equal to that of the extract used through the top of the condenser and immediately heat with a strong flame. If much amide nitrogen is suspected, heat for one-half hour after the yellow chlorine color disappears. Proceed for nitrate and ammonia nitrogen as previously directed.

NITROGEN BY DIGESTION WITH SULPHURIC ACID AND DIRECT NESSLERIZATION.—An aliquot, usually 10 ml., is digested with 10 ml. of concentrated sulphuric acid until colorless. The volume is then made to exactly 10 ml. with 50 per cent. sulphuric acid. An aliquot is then taken (0.5 to 2 ml. usually) and 20 ml. of water added. Litmus paper is added and the solution neutralized and Nesslerized as directed under procedure for ammonia nitrogen. This does away with distillation and titration as in the regular Kjeldahl procedure and lessens danger of losses. It also enables smaller amounts of N to be determined accurately. Note the following precautions:

(a). The appearance of cloudiness in the Nessler solution may be caused by several things:

(1) Solution too alkaline
(2) Too much NH_3 present
(3) Presence of too large amounts of sulphate
(4) Long standing
(5) Heat

Cloudy solutions must never be used for comparison in accurate work, although fairly accurate results may be secured in slightly cloudy solutions if the standard and unknown are made up under the same conditions and at

the same time. If proper precautions are taken and directions are followed clear solution will result. If extremely small amounts of NH_3 are being determined it is better not to use too large aliquots even if the color is light due to the introduction of too much sulphate.

(b). Of course, blanks must be run on reagents to make sure no NH_3 contamination is present. Sodium hydroxide solution can be freed of NH_3 by boiling.

DETERMINATION OF TOTAL NITROGEN IN PURE AMMONIA COMPOUNDS.—In order to determine whether the nitrogen in ammonium compounds could be estimated, the method was tried on ammonium sulphate, nitrate and chloride. Table I shows the results.

TABLE I

TOTAL N IN AMMONIUM COMPOUNDS

COMPOUND	THEORETICAL PERCENTAGE	PERCENTAGE AS NITRATE N	PERCENTAGE AS AMMONIA N	TOTAL N	PERCENTAGE ERROR
$(NH_4)_2SO_4$ ...	21.20	1.56	19.33	20.89	−1.46
$(NH_4)_2SO_4$ ...	21.20	1.25	20.58	21.83	+2.97
NH_4NO_3	35.00	17.85	17.52	35.37	+1.11
NH_4NO_3	35.00	17.60	17.42	35.02	+0.11
NH_4Cl	26.17	6.00	20.58	26.58	+1.57
NH_4Cl	26.17	4.81	21.82	26.63	+1.76

Fifty-milligram samples of the pure salt were oxidized as directed in the chlorate method (**1, 2**), the nitric acid determined by phenoldisulphonic acid and ammonia determined by Nessler's reagent as directed in the preceding procedures.

The results, except one, are slightly higher than theoretical. This shows that all the nitrogen is accounted for. In the case of NH_4NO_3 all nitrate was recovered and very little NH_3 oxidized.

It will be noted that a small amount of ammonia was oxidized in pure solutions. This was especially true with ammonium chloride. In order to see if this occurred in the presence of organic matter, sugar was added with the 50 mg. of NH_4Cl. The results were as follows:

SUGAR ADDED MG.	THEORETICAL PERCENTAGE OF N	PERCENTAGE OF N AS NO_3	PERCENTAGE OF N AS NH_3	TOTAL N DETERMINED
50	26.17	5.50	21.11	26.61
100	26.17	0.18	25.74	25.92
200	26.17	0	26.35	26.35
200	26.17	0	26.35	26.35

These data show that in the presence of sufficient organic matter no ammonia nitrogen is oxidized and hence the separation of nitrogen into

ammonia and other nitrogen is quantitative when enough organic matter is present.

DETERMINATION OF TOTAL NITROGEN IN ASPARAGINE.—Pure asparagine was tested to see what happened to the nitrogen in the chlorate oxidation. The results are shown in table II.

TABLE II

TOTAL N IN ASPARAGINE

COMPOUNDS	PERCENTAGE OF N AS NO_3	PERCENTAGE OF N AS NH_3	PERCENTAGE TOTAL N	THEORETICAL PERCENTAGE OF N
50 mg. asparagine	10.59	10.62	21.21	21.20
50 mg. asparagine	10.71	10.63	21.34	21.20
50 mg. asparagine + 50 mg. sugar	10.59	10.62	21.21	21.20
50 mg. asparagine + 50 mg. sugar	10.75	10.53	21.28	21.20
50 mg. asparagine + 100 mg. sugar		Incomplete oxidation		
50 mg. asparagine + 10 ml. H_2O. Acid through condenser	10.55	Not determined		

The data of table II show that a quantitative separation of the amino and amide nitrogen in asparagine was made and that all nitrogen was accounted for. Too much sugar resulted in incomplete oxidation.

This gives definite proof that the method may be used in grouping nitrogen compounds as previously discussed.

TABLE III

N IN PLANT EXTRACTS (MILLIGRAMS)

EXTRACT USED	N AS NO_3 BY CHLORATE METHOD (1)	N AS NH_3 BY CHLORATE METHOD (1)	SUM OF N AS NO_3 AND NH_3	TOTAL NITROGEN BY SULPHURIC ACID DIGESTION	
				DIRECT NESSLERIZATION	DISTILLED* AND TITRATED
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
10 ml. tomato	0.016	0.0413	0.0573	0.0410	.....
10 ml. "	0.016	0.0400	0.0560	0.0395	.....
10 ml. bean	2.70	2.45	5.15	4.88	4.89
10 ml. "	2.68	2.47	5.15	4.88	4.53
10 ml. bean†	2.63	2.52	5.15		

* Distillation and titration by HOWELL D. SPEARS of the Feed Control Department.

† Only 0.5 ml. was used in this case and the test was made by the rapid soluble N test (1).

COMPARISON WITH THE KJELDAHL METHOD.—In order to further check the method, nitrate-free tomato and bean tissues were secured by growing the plants in pure white sand for two weeks. The tissue was extracted with 2 per cent. acetic acid by adding a small amount of charcoal and grinding 3-gm. samples with 10 ml. of 2 per cent. acetic. The extracts were poured together until 50 ml. were obtained. The nitrogen was then determined by 4 different methods. The results are presented in table III.

The small amount of nitrogen in the tomato extract was due to true N deficiency. The quantity in 10 ml. of extract was too small to be estimated by the usual Kjeldahl procedure. The N in the bean extract came from the seed since the beans were used in the seedling stage. No nitrate nitrogen would be expected from the seed. Small amounts of readily oxidized nitrogen must have been present, however, since the Kjeldahl results are slightly low in the beans and considerably lower in the tomato. The total chlorate method and soluble methods check exactly, although the nitrate and ammonia nitrogen were slightly different. These results show that the chlorate methods gave very accurate results on the plant extracts tested. These methods seem to be especially advantageous where nitrate nitrogen is present and where a division into the major groups of nitrogen compounds is desired.

SOLUBLE NITROGEN TEST.—Tests were made on several compounds to see what part of the nitrogen was oxidized to nitrate by the soluble N test. Table IV gives the results.

TABLE IV

N BY SOLUBLE N TEST

COMPOUND USED	MG. N ADDED	MG. N AS NO_3 FOUND	COMPOUND USED	MG. N ADDED	MG. N RECOVERED
Sodium nitrate	0.05	0.0498	Aspartic acid	0.025	0.0254
Picric acid	0.05	0.0500	"	0.025	0.0250
Ammonium sulphate	0.05	0.0	"	0.025	0.0252
Asparagine	0.05	0.0250	"	0.025	0.0254
"	0.05	0.0250	"	0.025	0.0249
"	0.05	0.0244	"	0.025	0.02496
"	0.25	0.1250			
"	0.25	0.1255			
"	0.25	0.1248			
"	0.10	0.0500			

The data of table IV show that all nitrate, nitro, and amino acid nitrogen was recovered quantitatively, while ammonia and amide nitrogen was not obtained at all.

Tests were then made to see if Nessler tests would account for all the nitrogen. Table V shows these results.

TABLE V

INCLUSION OF NESSLER TEST IN SOLUBLE N TEST

COMPOUND USED	MG. N ADDED	MG. N AS NO_3	MG. N AS NH_3	TOTAL
Asparagine	0.05	0.025	0.02495	0.04995 (refluxed)
"	0.05	0.025	0.0106	0.0356 (not ")
"	0.05	0.025	0.02514	0.05014 (refluxed 10 minutes)
"	0.05	0.025	0.0058	0.0308 (not refluxed)
Aspagarine + 5 mg. sugar	0.05	0.025	0.0052	0.0302 (" ")
Aspagarine + 5 mg. sugar	0.05	0.025	0.02514	0.05014 (refluxed 20 minutes)
Ammonium chloride	0.05	0.0	0.05	0.050
" "	0.10	0.0	0.105	0.105

The results in table V show that amide nitrogen is recovered if the solution is refluxed and Nesslerized.

The soluble nitrogen test was further checked against the total chlorate method on N in tomato extract, with results shown in table VI.

TABLE VI

N BY TOTAL CHLORATE AND SOLUBLE TEST

EXTRACT	N AS NO_3	N AS NH_3	METHOD
	p.p.m.	*p.p.m.*	
Field tomatoes stopped by cold in fall	1110	1650	Total chlorate
	1020	1694	Soluble
Greenhouse plants	1040	1875	Total chlorate
	800	1852	Soluble

The methods checked almost exactly with the exception of nitrate N in the greenhouse plants. No explanation of why these did not check can be offered. The exact checks between these two methods in table III and the close checks with the Kjeldahl, however, offer proof that this was an experimental error of some kind and that the soluble N test is accurate. It was found that when rather large amounts of nitrogen are present, care must be taken to cool the solution before adding phenoldisulphonic acid and that it is best to double the amount of phenoldisulphonic acid added.

TOTAL NITROGEN BY COMPLETE CONVERSION TO NITRIC ACID.—The sample is treated as in the regular chlorate oxidation (**1, 2**) to the point where the yellow color begins to get light. Instead of stopping the refluxing then, another addition of about 0.5 gm. of sodium chlorate is made through the condenser and vigorous refluxing is continued. It is not necessary to remove

the flame while adding chlorate since the high temperature prevents ClO_2 formation. It is best not to let the solution cool before adding the chlorate. The chlorate addition is repeated whenever the yellow color becomes faint during the course of about one hour of refluxing. At the end of this time, if some chlorate is still present in the upper end of the condenser it may be necessary to flush it down with a little 50 per cent. sulphuric acid and boil until the color disappears and flush again to wash down nitric acid. The nitric acid formed is determined as in the regular procedure, by phenol-disulphonic acid.

Since, as was shown in table I, ammonium sulphate was the most difficult compound to oxidize it was tested by the above procedure. Twenty-one per cent. of N was found as nitric acid which is within 0.2 per cent. of theoretical. If some especially difficult substance is being determined an aliquot may be taken for colorimetric determination and if the conversion is thought not to be complete more chlorate can be added and the refluxing continued a while longer. If no increase is noted the oxidation is complete. If there is an increase the refluxing should be repeated until there is no more increase in the nitric acid. A record of the aliquots taken must be kept, in order to be able to compute the N content from the final readings, and added to the final figures.

NITROGEN IN PYRIDINE.—It has long been recognized that the Kjeldahl method will not determine the nitrogen in pyridine or quinoline. Tests were made on pyridine by the chlorate method and the following results were obtained:

MG. PYRIDINE	MG. N ADDED	MG. N AS NO_3	MG. N AS NH_3	TOTAL MG. N FOUND
50	8.86	3.71	5.10	8.81

These results were obtained only by prolonging the oxidation period considerably, as follows:

50 mg. of pyridine and 1 gm. of sodium chlorate were placed in a 200-ml. Erlenmeyer flask and 25 ml. of 50 per cent. by volume sulphuric acid were added. The flask was attached to an efficient reflux condenser and refluxed until the yellow color was nearly gone. The heat was removed, more chlorate added (0.5 gm.) and the flask was again heated until the yellow color had nearly disappeared. This was repeated for almost 1 hour. After the yellow color had disappeared, the condenser was washed out with 10 ml. of 50 per cent. H_2SO_4 and the nitrate and ammonia N determined in the regular ways. This extra refluxing is not necessary for most samples but was necessary in this case.

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DETERMINATION OF CONSTANTS FOR CURVES OF WATER ABSORPTION BY DRY ORGANIC SUBSTANCES

CHARLES A. SHULL AND S. P. SHULL

Some years ago the writers engaged in a study of water absorption by dry organic matter, such as seeds (**1**) and gelatin disks (**2**), with special reference to the temperature coefficient of water absorption by seeds (**1, 3**). It was found not only desirable but necessary to study the absorption data mathematically, and to attempt to refine the methods of studying the rate of water intake so that mathematical computations could replace cruder methods of measurement. After a study of all types of curves, it was found that the best results could be obtained by use of an equation having the general form: $y = a \log (bx + 1) + c$. Using this equation we were able to pass curves through the data of observation with gratifying closeness.

At the time this work was done, it did not seem necessary to stress the details of the mathematical processes. The calculated curves were needed only to insure accuracy; how they were obtained did not seem important. Nothing was said, therefore, about the manner in which the arithmetical values of the constants *a, b,* and *c* of the given equation were derived. During the years intervening since this work was done, a number of inquiries have been received asking for information as to just how the values of the constants were obtained. In presenting the method briefly, we are deeply conscious of the fact that this discussion is purely mathematical, not physiological; the inquiries, however, come from plant physiologists. We hope it is not inappropriate, therefore, to supply the information through a journal which reaches those who seem most interested, and who may be most helped in connection with other studies of rates that change with time, if they happen to require the use of the same general equation.

Before taking up the problem of approximation in connection with the constants, it seems advisable to point out a few fundamental mathematical facts. If one selects *only three* data points through which to pass a curve, it is possible with an equation of three constants to pass any type of curve—circle, ellipse, parabola, or hyperbola—through the three points. In selecting the three data points out of a long series of observations, one may easily make a poor choice of one or more points. That is, the points selected for a trial curve may lie above or below the line of the final accurately fitted curve. If any point lies outside of this "best curve" it is a necessary and vital part of the analysis to discover such errors, and to correct for them by changing the constants which determine the position and path of the finally accepted curve. While a part of the process may seem like "trial and error," it is always possible to discover how much correction must be applied to which

constant to swing the curve into a new position, or to change its shape in some desired direction.

We feel that the simplest way to explain the procedure is by use of a concrete example taken from one of the earlier absorption studies, and to show just how the equation $y = 29.6 \log (0.0078x + 1) + 4.38$ (see **3**, p. 266, table II) came to have the values 29.6, 0.0078, and 4.38 for the constants *a*, *b*, and *c*, respectively.

Given the equation: $y = a \log (bx + 1) + c$,

To determine the value of the constants *a*, *b*, and *c*, in a specific series of observed data to which this type of equation applies.

Since the method of approximation must be employed, the problem is first changed to a form involving only one of the constants.

To eliminate *c*:

$$y_1 = a \log (bx_1 + 1) + c \tag{1}$$

$$y_2 = a \log (bx_2 + 1) + c \tag{2}$$

Subtracting equation (1) from equation (2),

$$y_2 - y_1 = a \log (bx_2 + 1) - a \log (bx_1 + 1), \text{ or}$$

$$y_2 - y_1 = a \log \frac{bx_2 + 1}{bx_1 + 1} \tag{A}$$

In this manner constant *c* is eliminated from the equation.

The constant *a* may also be eliminated from the equation. In order to do this, it is necessary to have a second equation having only *a* and *b* as constants. An independent equation similar to (A) is readily obtained:

$$y_3 = a \log (bx_3 + 1) + c \tag{3}$$

Subtracting equation (2) from equation (3),

$$y_3 - y_2 = a \log \frac{bx_3 + 1}{bx_2 + 1} \tag{B}$$

Eliminate constant *a* by dividing (B) by (A):

$$\frac{y_3 - y_2}{y_2 - y_1} = \frac{\log \dfrac{bx_3 + 1}{bx_2 + 1}}{\log \dfrac{bx_2 + 1}{bx_1 + 1}} \tag{C}$$

In equation (C) the values of y_1, y_2, y_3 are known ordinates corresponding to certain known values of x_1, x_2, x_3. We may now substitute these known values in equation (C) and carry out the operations to obtain the value of constant *b*, the only unknown quantity in the equation.

It is then easy, with *b* known, to substitute its value in equation (A) in which *a* becomes the only unknown quantity. With *a* known, the value of *c* may finally be found by substituting the determined values of *a* and *b* in equations (1), (2), or (3). To obtain most accurate results one should use six-place logarithms in making these computations.

The preceding steps present, in their simplest form, the mathematical steps involved in any case. The work may be carried to such a degree of refinement as to make the curve pass very precisely through any three points chosen for the calculations.

These values of the constants are to be regarded as approximations only. The reason for this is obvious; for in accumulating any given mass of data, there are many ways in which errors of observation may arise. These errors are inherent in the work, and are not entirely attributable to the investigator. Any value of y may be slightly above or below the true position of the curve that passes through the data with satisfactory precision. Some of the causes of irregular values of y have been considered in a previous paper (**4**). One is most likely to find, therefore, that the curve which passes through the three points chosen for the approximation may not pass through any other points in the entire series of observations, and not even satisfactorily close to them. In other words, this preliminary curve may afford a very poor "fit" to the data of observation. In the event that a very poor fit is obtained by the preliminary work on the constants, it becomes necessary to change the values of *a*, *b*, or *c*, or of all of them, in such manner as to bring the curve into satisfactory agreement with the observations. The general problem is to swing the curve, or change its form just enough, to give it a location which represents a fair compromise with the irregular data, leaving some points above the curve, and others below the curve, with all points as close to it as possible.

In order to make such changes in the value of the constants intelligently, it will be well briefly to examine the effects of changing the values of each of the constants. The constant *a* determines in large measure the general upward rise of the curve from left to right. If it is found that the original curve from the three chosen points extends downward to the left *below* the observational data points, and rises *above* them to the right, the needed correction is obtained by *decreasing* the value of *a*. If the position of the curve is reversed with reference to the data as a whole, *i.e.*, too high at the left and too low at the right, the value of *a* must be *increased*. These changes tend to swing the curve into a position parallel with the data, although the curve may then lie *too low* or *too high*, even if it is parallel; for the change of *a* also displaces the curve *upward* or *downward* as it is increased or decreased.

After *a* has been adjusted to give a curve sensibly parallel with the line of data points, the whole curve is lifted or lowered into proper position by increasing or decreasing the value of *c*.

If, when the curve has been satisfactorily located at both left and right, the middle portion of the curve rises too high (above the compromise line of data points) the value of *b* must be decreased, and then new values of *a* and *c* must be determined. Or if the central region of the curve is too low, the value of *b* must be increased, and the necessary changes made in *a* and *c*. In

other words, changes in b change the radius of curvature, so that it is flatter, or more arched, as b decreases or increases.

Long experience with these problems leads us to suggest the following procedure as the best one to use in such work:

On accurately ruled coordinate paper, plot with the greatest possible accuracy all of the data points as determined by their abscissae and corresponding ordinates. Examine these along the entire line, to note any points that are obviously or apparently above or below a smooth curve through their midst. Choose values of y_1, y_2, and y_3 not suspected of being too high or too low—one of them near the left end, another near the right, and a third somewhere between, preferably nearer to the first than to the last point. Use these three values of y, with their abscissae values of x, in determining the value of b in equation (C). The value of b so determined is not likely to need change, but it may need changing, as in the following concrete example. The values of a and c, on the other hand, often have to be changed slightly to effect a satisfactory compromise with the data.

Example

To illustrate the method, now, as a concrete problem in experimental work, we may take water absorption data obtained with seeds of Hickory King corn at a constantly maintained temperature of 35° C. The following data appear in a previous paper (3, p. 265, table I).

Absorption period	Water intake (air dry basis)
min.	%
15	5.76
30	7.10
45	8.34
60	9.31
90	11.09
120	12.85
180	15.67
240	17.95
300	19.87
420	23.21

Using time values as abscissae and percentage values as ordinates, the successive data points are plotted on accurately ruled coordinate paper. Examination of the plotted points shows that the 90-min. percentage is too low, and the 420-min. point too high with reference to the rest of the data. All other points lie on or near a smooth curve. We may now select three of these points for a tentative determination of the value of constant b, as follows:

$$x_1 = 15,\ y_1 = 5.76$$
$$x_2 = 120,\ y_2 = 12.85$$
$$x_3 = 300,\ y_3 = 19.87$$

Then the left side of equation (C) by substitution of these values, becomes:

$$\frac{y_3 - y_2}{y_2 - y_1} = \frac{19.87 - 12.85}{12.85 - 5.76} = 0.9901 +,$$

and the right side of equation (C),

$$\frac{\log \dfrac{bx_3 + 1}{bx_2 + 1}}{\log \dfrac{bx_2 + 1}{bx_1 + 1}} = \frac{\log \dfrac{300b + 1}{120b + 1}}{\log \dfrac{120b + 1}{15b + 1}}$$

If, now, we choose some value for b, as 0.007, and perform the mathematical operations of this last equation, the resulting value is 1.0237 –. This is greater than the true value, 0.9901 +, just obtained from the left side of equation (C). If we put $b = 0.008$, the resulting value is 0.9843 +, which is now less than the true value. Obviously the value of b lies between 0.007 and 0.008, and much nearer to the latter than to the former. If b is taken as 0.0078, the resulting value for the right side of equation (C) is 0.9916 +. This value is again slightly greater than the true value, consequently the value of the constant should be somewhat greater than 0.0078. If we put $b = 0.00784$, we now find that the value of the right side of equation (C) (last equation above), is 0.9901 –, a very close approximation to the true value of b obtained from the left side of equation (C).

Having obtained the constant b, substitute its value in equation (A) or (B), and the value of constant a is found to be 29.58 –. We can now substitute the values of both a and b in equation (1), (2), or (3) and determine the value of constant c, which is found to be 4.332. With these three values for the constants ($a = 29.58$, $b = 0.00784$, and $c = 4.332$) the curve now passes very precisely through the three chosen points (x_1, y_1), (x_2, y_2), and (x_3, y_3).

Using the formula $y = 29.58 \log (0.00784\,x + 1) + 4.332$ we may now compute the value of y for each value of x in the data given. These computed values are then set out in a horizontal line to represent the true curve. The three values used in the preceding computations agree exactly, of course, with the experimental data, since the curve was developed specifically for these three points. Above or below each computed y value of the curve, as it happens to lie, we write the corresponding observed value. In this 35° C. absorption series we find:

		7.10	8.34	9.31			15.67	17.95		23.21	
Computed,	5.76—	7.05—	8.21—	9.28—	11.19—	12.85—	15.64—	17.93—	19.87—	23.05	
					11.09						

Most of the data of observation lie slightly above the curve, so it is obvious that the curve is somewhat too low, particularly at its left end. The choice

of 5.76 (x_1, y_1) as one of the points in the curve has depressed that end of the curve, indicating that 5.76 per cent. is a low reading for the 15-minute value of absorption at 35° C. with the variety of corn used.

To raise the curve at the left end without changing the other chosen points (12.85 and 19.87) reduces the convexity of the curve as a whole, and requires a decrease in the value of b, which we said was not likely to need change. At a venture, the value of b is reduced to 0.0078. Now the new values of a and c must be determined by the methods already outlined, and a new set of y values computed for the entire series.

As the value 5.76 is being abandoned as a correct starting point for the curve, we substitute 0.0078 for b in equation (B)—not in (A)[1]—and find $a = 29.64$. Now putting $a = 29.64$, and $b = 0.0078$ in equation (2) or (3)—not in (1)[1]—we find $c = 4.346$. Now proceeding to compute the new values of y for comparison with the observational data, we find:

		7.10	8.34	9.31				15.67	17.95			23.21
Computed,	5.77—	7.05—	8.22—	9.29—	11.19—	12.85—	15.64—	17.93—	19.87—	23.05
	5.76				11.09							

The convexity of the curve has been slightly reduced, but it still lies too low. One can see, also, that the calculated and observed values are closer together at the right than at the left. The general slope must be reduced by lifting the left end of the curve. This is accomplished by decreasing the value of a from 29.64 to 29.6. The corresponding value of c, derived now from equation (2), is 4.36. Again calculating the y values (with $a = 29.6$, $b = 0.0078$, $c = 4.36$) we obtain the following series:

		7.10	8.34	9.31				15.67	17.95	19.87	23.21
Computed,	5.78—	7.06—	8.23—	9.30—	11.20—	12.85—	15.64—	17.92—	19.86—	23.04
	5.76				11.09							

The total effect of changing a has been to raise the left end of the curve by a small amount, and to depress the right end by a small amount, so as to make the curve now sensibly parallel to the observed data; but the curve is still too low, with seven values of the observations above, and only two below the computed values of the curve. The computed curve lies too low because it was *assumed* that the points 12.85 per cent. and 19.87 per cent. were without error of observation when the three original values were selected for the establishment of the curve. As it turns out, these values were too low by about 0.02. If we add this amount to the value of c (to lift the entire curve), we have $c = 4.38$. A final computation of the y values now yields the following series:

		7.10	8.34					15.67	17.95			23.21
Computed,	5.80—	7.08—	8.25—	9.32—	11.22—	12.87—	15.66—	17.94—	19.88—	23.06
	5.76			9.31	11.09	12.85				19.87		

This distribution of the observations with reference to the computed

[1] Because y_1, which occurs in both (A) and (1), has been abandoned as too low.

values, five above and five below, is satisfactory, and nothing would be gained in any further attempts to adjust the constants. The curve accepted for this series of data, then, becomes $y = 29.6 \log (0.0078x + 1) + 4.38$.

The development of a considerable number of such curves involves much mathematical tedium unless one enjoys doing it. In accurate analyses it may be necessary, whether one enjoys it or not. We have tried to illustrate the derivation of the original constants, whereby approximate values may be obtained for the testing of fit. And we have shown how, by small changes first of b, then of a, and finally of c, the curve may be fitted, as accurately as the data permit, to the observed values. Such closely fitted curves are ideal for use in the computation of the rate of the process under investigation at any given moment within the limits of the curve.

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ELECTRICAL RESPONSE OF *PHASEOLUS MULTIFLORUS* TO ELECTRICAL CURRENTS

W. S. Rehm

(WITH FOUR FIGURES)

Several investigators have studied electrical polarization phenomena in multicellular plants. Marsh (**1**) found in the onion root and Rosene (**4**) in branches of the Douglas fir that the effect of an applied current was dependent on the direction of the current flow. These investigators reported that applied potentials opposing the inherent potentials resulted in a temporary increase in the magnitude of the inherent potentials, while potentials applied in the same direction as the inherent ones produced a temporary decrease in magnitude. The present paper is concerned with a report of the effects of applied current on the inherent potentials of *Phaseolus multiflorus*. It was found as will be demonstrated in the following pages that, in general, the gross response to applied current in this particular plant is independent of the direction of current flow.

Methods

The method for measuring the potentials and the conditions under which the experiments were performed were essentially the same as those described in previous papers (**2, 3**). Figure 1 illustrates the method of applying the current and measuring the potentials. In this diagram X and Y represent

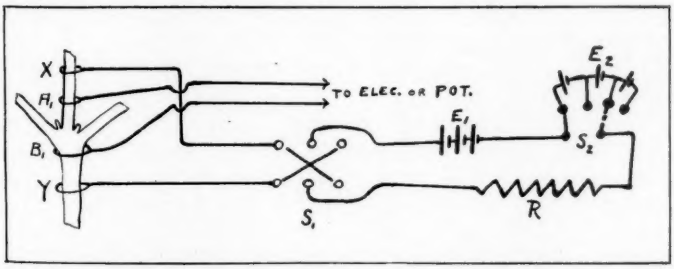

Fig. 1. Method of applying current. X and Y are contacts through which the current is sent. A_1 and B_1 are contacts between which the potential difference was measured. Elec. refers to electrometer and Pot. to potentiometer. For further explanation see text.

the contacts through which the current was applied and A_1 and B_1 represent contacts from which the inherent potentials were measured. E_1 represents a "B" battery of appropriate voltage and E_2 represents a series of 1.5-volt dry cells. By means of switch, S_2 the number of dry cells included in the circuit could be varied. R represents a large resistance (2 megohms). The

purpose of this large resistance was to prevent fluctuations in resistance of the plant from affecting appreciably the magnitude of the current flowing through the plant. S_1 is a double-pole double-throw switch used for reversing the current.

It is to be noted that separate electrodes ($Zn : ZnSO_4$) were used for measuring the potentials, which eliminates the possibility of errors from polarization effects at the electrodes.

In all the experiments reported in this paper the current was passed through the plant for 3-minute periods. Owing to the relatively long period of the instruments employed the first readings were not obtained until 30 seconds after turning off the current. For a more detailed analysis of these phenomena a different type of instrument must be used.

Results

The following figures represent typical responses and in each type of experiment at least 5 plants were used. Figure 2A shows the effect of

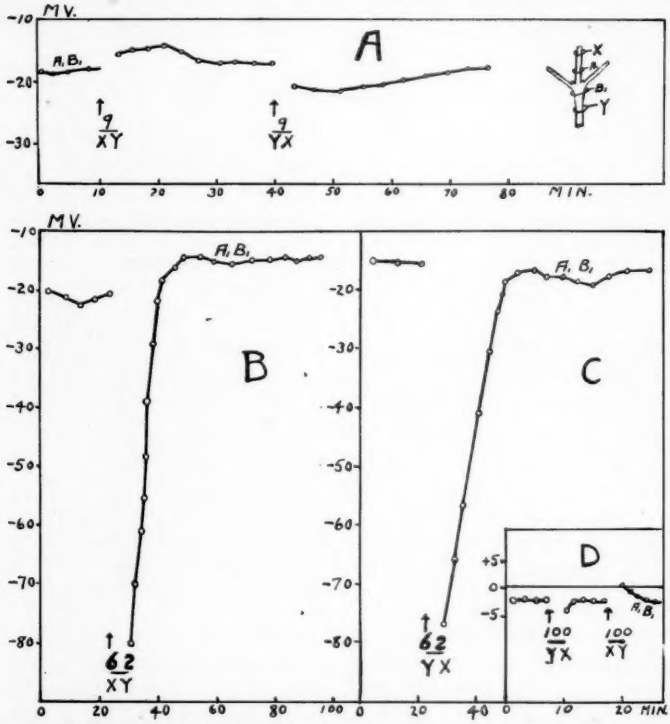

FIG. 2. Abscissa in millivolts. Ordinate, time in minutes. X was 2 cm. above A_1. Y was 2 cm. below B_1. The diagram in A showing the position of the contacts applies also to both B and C.

relatively small currents on the potential difference from A_1 to B_1 (referred to hereafter as A_1B_1). At the time indicated by the first arrow, a current of 9 microamperes was sent from X to Y; and at the time indicated by the second arrow, a current of the same magnitude was sent in the opposite direction from Y to X. The current from X to Y caused a temporary decrease in the absolute magnitude of the potential A_1B_1 while sending the current from Y to X resulted in a temporary increase in the magnitude of the potential.

On the other hand, currents well above the threshold value produced a marked increase in the magnitude of the potentials irrespective of the direction of the current.

In figure 2 B and C, currents of 62 microamperes were sent from X to Y and from Y to X. In both plants there was a large increase in the magnitude of the potential A_1B_1. After sending the current from Y to X, opposing the inherent potential, the potential A_1B_1 was maintained at a slightly greater magnitude than before the application of current, whereas after the current passed from X to Y the potential A_1B_1 was maintained at a lower magnitude (*i.e.*, nearer the base line). The potentials returned very gradually to their original magnitude. In fact this return was so slow that spontaneous variations of the potential prevented any very accurate determination of the time interval involved.

That this effect is dependent on the living cells is demonstrated by figure 3D which shows the effect of a current of 100 microamperes on the node potential A_1B_1 of a plant killed by immersing it over night in a formalin solution. In this case the effects were of small magnitude and depended on the direction of the current. When the current opposed the inherent potential, the magnitude was slightly increased; and when it was applied in the same direction as the inherent potential the latter was decreased.

The typical response of the petiole potentials to an applied current is shown in figure 3. This figure shows the effect of currents of 2, 6, and 30 microamperes on the potentials O_1P_1 and $O_1'P_1'$. In each case the current

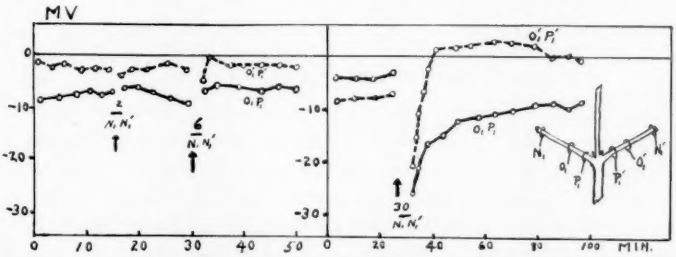

FIG. 3. N_1 and N_1' were placed at the apex of the petioles, O_1 and O_1' were placed 1.5 cm. above P_1 and P_1'.

passed downward from N_1 to P_1 in the one petiole, and upwards from P_1' to N_1' in the other petiole. If the response to the applied current should depend on the direction of the current, then the response of O_1P_1 would be different from that of $O_1'P_1'$. Two microamperes produced practically no observable effect while 6 microamperes produced an observable effect dependent on the direction of the current. However with 30 microamperes the primary effect was independent of the direction of the current. A large increase was produced in the magnitude of both potentials. The potential $O_1'P_1'$, after returning to a relatively constant level, was less negative than before, whereas O_1P_1 was more negative than before.

Figure 4A shows the effect on the 3 node potentials [A_1B_1, A_1P_1, and $A_1'P_1'$, see previous paper (**2**)] of sending the current from O_1' to X. In

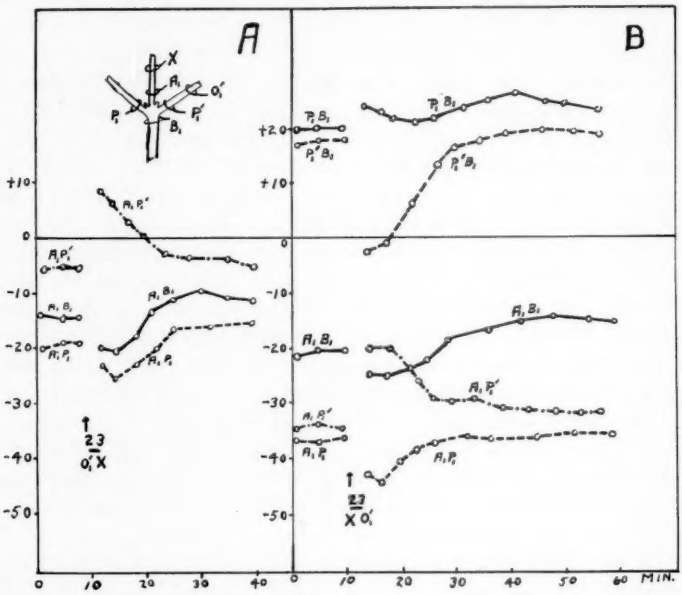

FIG. 4. X was placed 2 cm. above A_1, and O_1' was 2 cm. above P_1'. The diagram in A also applies to B.

this experiment A_1B_1 became more negative (an increase in absolute magnitude) as might be predicted from the results presented in figure 1 since the current flowed through most of the region from A_1 to B_1. A_1P_1 shows a change similar to A_1B_1 which again would be expected since the region through which the current flowed is roughly common to both A_1P_1 and A_1B_1. The response of the potential A_1P_1', although it decreased in magnitude, is not actually an exception to the general rule, as the following

analysis demonstrates. The response of A_1P_1' is made up of two components, namely A_1 to the node and P_1' to the node. Since both of these responses should result in an increased basal positivity they would be oriented in opposite directions and the effect on A_1P_1' would depend upon the difference of the two effects. Figure 4B shows the response of the node potentials plus those of $P_1'B_1$ and P_1B_1. Since the response of $P_1'B_1$ is greater than that of A_1B_1 the direction of the response of A_1P_1' ought to be determined by $P_1'B_1$ which is actually the case. An inspection of the figure shows that the effect on A_1P_1' is a quantitative summation of the effects on $P_1'B_1$ and on A_1B_1.

In this figure P_1B_1 shows an increased apical positivity which was a typical response of this potential to current sent in either direction through the main stem and opposite petiole.

Discussion and conclusions

The results presented in this paper demonstrate that in *Phaseolus multiflorus* relatively small currents produce an effect that is dependent on the direction of the current, while currents of greater magnitude produce in the main axis and petioles an increase in basal positivity. In a former paper (**3**) it was demonstrated that decapitation produced a similar increase in basal positivity. Both results demonstrate a latent oriented mechanism, inherent in the plant, capable of producing comparatively large electrical potentials. This is roughly comparable to the situation in nerve and muscle. In these tissues stimuli produce an electrical response that is determined by an inherent oriented mechanism. The analogy suggests that this electrical response may play a part in the economy of this plant.

The writer has demonstrated in previous work (**2, 3**) that in *Phaseolus multiflorus* there is an intimate relation between the orientation of bioelectric potentials and morphology. When the plant changes from one morphological state to another there is a concomitant reorientation of electrical potential patterns. Interestingly in this connection, the main electrical polarity of *Phaseolus multiflorus* was one of basal positivity.

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TRANSPORT OF INORGANIC IONS IN POLAR PLANT TISSUES

F. W. WENT

In a short note some remarkable parallelisms between the polar auxin transport in stems and salt accumulation by many plant cells were pointed out (**8**). It was suggested that both phenomena were an expression of the same basic property of active cells, namely, their adenoid activity. In this connection the question arose whether inorganic salts were transported inside plant organs by the same mechanism which causes the auxin to move from apex to base only. Some phenomena seemed to support this idea of polar movement of bases and acids in general. The excretion from roots is acid whereas the exudate from leaves is basic.

To get data on the polar movement of inorganic ions inside stems it was deemed necessary to apply and test for exceedingly small amounts; for experiments of WENT and WHITE (**9**) had indicated that the polar transport mechanism moves very small amounts of auxin only: in *Avena* coleoptiles not more than 10^{-5} mg. of indoleacetic acid is transported from apex to base per mm.^{2} per hour. Thus quantities of the same order of magnitude should be determinable of each ion to be tested. Since it is possible to induce such strong radio-activity in certain elements commonly found in plants, that amounts of less than 10^{-5} mg. can be measured with a Geiger counter, it was decided to try the transport of some radioactivated elements in polar plant tissues. So far these experiments had not been carried out under exclusion of the transpiration stream (**3**). Through the courtesy of the divisions of Physics and Plant Nutrition of the University of California, and especially through the kind help of Mr. P. R. STOUT and Dr. F. SKOOG, the writer was enabled to carry out some experiments in Berkeley.

Different samples of radioactive bromine as Br^-, radioactive phosphorus as PO_4^- and radioactive sodium as Na^+ were tested on *Avena* coleoptiles and *Helianthus* hypocotyls and epicotyls. All transport experiments were carried out in a physiological dark room kept at 25° C. and 85 per cent. humidity, in which also the auxin determinations were made. The radioactivity was determined in an adjoining room with a Geiger counter in which the samples could be brought in very close proximity to the electrodes. The samples were wrapped in cellophane to prevent contamination of the counter surface. Of each sample, readings were taken at 1-minute intervals for periods of at least 5 minutes to get an idea of the significance of the values measured. This was especially necessary when the counts ran close to the "background."

Two types of experiments were carried out. The first and simpler one was less satisfactory. Either to the apical or basal cut surface of a cole-

optile or hypocotyl a block of agar soaked in a very dilute solution of the radioactive material was applied. After 3 to 6 hours the stems were cut into segments of 3 or 5 mm. length, and the radioactivity of each section was determined. This procedure had several disadvantages. In the first place the possible accumulation of the ions by the cells made a clear interpretation of the results rather difficult. And in the second place it was not known whether the auxin transport was polar at the time of ion transport, although in a control experiment done with the same material in the same darkroom the transport of indoleacetic acid was perfectly polar. The results of this type of experiment did not show any consistent preferential direction in the transport of Br^- and Na^+ in *Avena* coleoptiles and *Helianthus* stems.

The most significant results were obtained with the following technique. Agar blocks of standard size ($2.5 \times 2.5 \times 1.5$ mm.) were soaked in the radioactive solutions. Then 5-mm. sections of coleoptile or hypocotyl were placed on them, either with their base or with their top down (it has been shown by various authors, *e.g.* (**5**), that gravity does not influence the polar longitudinal auxin transport). On each section was then placed another standard agar block soaked in a solution of 5 mg. indoleacetic acid per liter of water. After 3 to 6 hours the lower blocks were tested for auxin and the upper blocks for radioactivity. In this way the polarity of auxin transport was determined in the same sections simultaneously with the inorganic ion movement. In every one of the 120 sections tested for auxin the polarity was absolute: no trace of indoleacetic acid was found when it had been applied from the base, whereas strong curvatures were obtained when the basal block was analyzed after application of auxin from the apical cut surface of the section. Thus the traces of radioactive material had not influenced the physiological behavior of the tissues.

In table I the results of these experiments are summarized. The figures give the number of counts per minute, from which the background has been subtracted. Therefore, below about 1000, the concentration of the radioactive ion is directly proportional to the figure given. Any figure above 4 can be taken to indicate the presence of significant amounts of radioactive material. At higher values the standard deviation amounts to about 4 per cent.

Although it makes little difference for the indoleacetic acid transport, whether the tissues are cut nearer the apex or the base of the coleoptile or hypocotyl, in the inorganic ion transport we observe a consistent and significantly larger amount moving through basal than through apical parts of the same organ. The differences are especially marked for the PO_4^- movement. Care was always taken, therefore, to compare sections from corresponding regions of the plant, and the transport through more apical and

TABLE I

RADIOACTIVITY IN COUNTS PER MINUTE OF THE UPPER AGAR BLOCK, WHICH WAS SEPARATED FROM THE LOWER AGAR BLOCK CONTAINING THE RADIOCTIVATED MATERIAL BY A 5-MM. SECTION CUT FROM 8-DAY-OLD HELIANTHUS HYPOCOTYLS. WHERE MARKED + THE POLARITY OF THE AUXIN TRANSPORT WAS CHECKED WITH ALL 24 SECTIONS OF THE TRANSPORT EXPERIMENT

Transport from ⟶					Apex to base		Base to apex	
Compound	Concentration	Number of sections	Time of transport	Polarity of auxin transport	Apical sections	Basal sections	Apical sections	Basal sections
	gm./l.	*section*	*hr.*					
Na^+	0.5	3	3½	+	88 89	156 97	81 99	97 105
"	"	6	4½		57	85	109	180
"	0.05	6	2¾		19	19	13	17
"	"	6	5	+	15	11	22	11
"	"	6	6½		47	65	59	30
"	0.005	10	5		5		3	
Na^+	Mean				52	72	64	73
PO_4^-	0.8	6	2¾	+	4 ± 1.4	75 ± 2.7	13 ± 1.2	97 ± 3.5
"	"	3	5		1 29	62 74	20 31	84 139
PO_4^-	Mean				11	70	21	107
Br^-	0.2	6	3¾	+	2	4	3	3
"	"	11	3¾	+	9		5	
"	"	11	4		10 ± 3.1		8 ± 1.7	
"	"	12	6½		1		7	
Br^-	Mean				5		5	

more basal regions was determined separately. But no clear evidence of polarity in the inorganic ion translocation could be detected. Only in the case of the most apical hypocotyl sections some indication of polarity was found, but this is not sufficient to account for polarity in general. As mentioned before, *all* parts of the coleoptile and hypocotyl have an equally pronounced polarity in their indoleacetic acid transport, so that in *all* sections a polar inorganic ion transport had to be expected if transport of the two types of substances were caused by the same mechanism. Although quantitatively the experiments leave many questions to be answered, one conclusion seems well established. *In the same tissues simultaneously there occurs a polar auxin transport and a non-polar movement of inorganic ions.* Thus there is no escape from the fact that the movement of auxin and of Na^+, Br^- and PO_4^- is controlled by completely different mechanisms. On different grounds CLARK (**2**) came to the same conclusion, since sodium glycocholate completely inhibited polar auxin transport while it had no influence on the accumulation of Br^- by barley roots.

One may envisage this as a fundamental difference in the locomotor mechanism for the movement of these substances, or simply as a difference in the path of transport. If (**1**) we accept VAN DER WEIJ'S notion (**6**) of auxin movement in the surface layer between protoplasm and cell wall, where an adsorbed film of auxin would move in the manner exemplified by VAN DEN HONERT'S model (**4**) and if (**2**) the polarity of the auxin transport were located somewhere along this surface layer, then only substances which positively adsorb on this boundary layer could be transported in a polar way. Since auxin a, indoleacetic acid and similar growth-promoting substances are lipoid soluble and will positively adsorb on a water-air interphase, whereas the ions tested lack both these properties, the latter could not move according to a VAN DER WEIJ-VAN DEN HONERT scheme.

Whatever explanation one accepts for the discrepancies between the mode of transport of auxins and inorganic ions, it is clear that the accumulation mechanism for inorganic ions and the polar transport mechanism for auxin are not identical, and must be located in different parts of the cell. Another discrepancy between these two mechanisms may be pointed out. The cell is able to accumulate both anions and cations. The rather meager experimental evidence (**1, 7**) however, indicates that in the polar transport mechanism all acids which were tested moved from apex to base and all bases from base to apex. Further work, including many more substances, which must be measurable in minute quantities, will have to confirm this conclusion.

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THE ESSENTIALITY OF CERTAIN ELEMENTS IN MINUTE QUANTITY FOR PLANTS WITH SPECIAL REFERENCE TO COPPER

D. I. Arnon and P. R. Stout

The recent discoveries of the importance of small amounts of boron, manganese, copper, and zinc (**1, 2, 4, 7, 13**) in the physiology of higher plants, and in a number of soil-plant problems of considerable agricultural importance, have added interest to studies on the rôle of these elements in plant nutrition. The most obvious and important question is whether or not these elements are indispensable to the growth of plants.

Recent investigations in this laboratory (**12**) on the importance of certain metals in minute quantity in the economy of higher plants, have resulted in the development of an experimental technique which makes possible a consistent and reproducible demonstration of the essentiality of copper, manganese, and zinc for the growth of higher plants. Although the details of the procedures will be described elsewhere (**12**) it is desired to present at this time some considerations and conclusions which may be of general interest.

It was deemed desirable to test experimentally the essentiality of each element according to the following criteria: an element is not considered essential unless (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium. From that standpoint a favorable response from adding a given element to the culture medium does not constitute conclusive evidence of its indispensability in plant nutrition.

Conclusive proof of the indispensability of the so-called microelements, that is, elements required in minute amounts by plants, can be obtained by the use of artificial culture media (the water culture technique was used in this investigation), provided special procedures are employed to remove incidental impurities. The physiological importance of these contaminants is illustrated by the finding that young tomato plants grown in Pyrex glass containers with nutrient solutions deficient in zinc gave a measurable response to the addition of 1 gamma (0.001 mg.) of zinc to a plant (this gave a zinc concentration in the culture solution of 5 parts per billion). This response to a minute amount of zinc as well as similar responses to copper and manganese were consistently obtained only when redistilled water and purified chemicals were used. The metal content of ordinary distilled water was reduced by redistillation, using

a Pyrex glass condenser, from 10 to 100 to consistently less than 0.2 part per billion. The metal impurity derived from salts was reduced, by using a modification of STEINBERG's procedure (**11, 12**) from 15 to 150 to consistently less than 0.1 part per billion.

The experimental demonstration of the essentiality of microelements for plant life is frequently rendered difficult not only because plant needs may be satisfied by incidental contaminants, but also because different species vary greatly in their requirements. In this laboratory, for example, it was repeatedly found that a standard culture solution to which no manganese or boron was added produced excellent barley plants but gave severe boron and manganese deficiency symptoms in tomato plants. It may be advantageous therefore to select for experimental purposes plants having relatively high requirements for the microelement studied. For any one species the onset of deficiency symptoms may be hastened by reducing the reserve in the seed, either by using seed obtained from plants grown with a limited supply of microelements (**5**) or by early removal of the seed from the seedling (**10**). Thus the extent to which it is necessary to purify the culture medium in order to produce deficiency symptoms may be reduced through a suitable selection and preparation of experimental plants.

Since it cannot be assumed that any purification procedure has completely removed a given element from the nutrient medium, the conclusions from a negative result should best not be drawn with finality. When an element is omitted from a carefully prepared and purified artificial culture medium without seemingly hindering the growth and reproduction of plants, it need not be concluded that it plays no essential function in the physiology of plants. Regardless of the care exercised in the preparation of the culture medium, a minute, but physiologically significant, impurity may have persisted and needs to be considered as well as the reserves contained in the seed. It would seem best in such cases to determine analytically, whenever possible, the upper limit of impurity that may be present in the culture medium, and express the results of the experiment on a quantitative basis; that is, that with a certain upper limit of supply of a given element, no response is obtained with certain species of plants. It is possible that the attainment of a greater degree of purification of the culture medium by means of improved experimental procedures, the use of seeds having a low content of, or the selection of plants having a high requirement for, a particular element may give different results.

If these views are accepted, there can be no *a priori* objection to regarding almost every element in the periodic table, and particularly those most frequently encountered in plant products, as susceptible of being shown at some time to be essential for plants. (This is in accord with similar views expressed by HOPKINS (**3**).) What can be stated definitely at the present time

is that a given element is essential or not, depending on whether the plant requires it in an amount greater than that present in the culture medium as a result of contamination. It was found, for example, that if the combined zinc, copper, lead, cadmium, and mercury content in the nutrient solutions was reduced to less than 0.1 of a gamma (0.0001 mg.) to a plant, as determined by analyzing the culture solution (**12**), very severe deficiency symptoms were obtained. In this case a definite response was obtained from supplying 2 gammas of copper and 2 gammas of zinc, but no further improvement was produced by supplying to a plant 0.5 of a gamma each of lead, cadmium, and mercury. These results, while showing the indispensability of zinc and copper for the growth of tomatoes in amounts larger than those left behind in the purified culture medium, were interpreted as permitting no final conclusion as to the rôle of cadmium, lead, and mercury in the nutrition of tomatoes. The possibility that these elements may be required in amounts smaller than the incidental impurities which could not be removed from the culture solution by the present technique, cannot be *a priori* excluded.

This quantitative approach to the problem of essentiality of microelements is regarded not as a mere theoretical generalization, but as a point of view conducive to a search for more refined analytical methods and procedures for growing plants, which would make it possible to investigate the rôle of a number of new elements in plant nutrition.

After experimentally producing the characteristic deficiency symptoms for an element, the next step was to show that it had a specific and direct effect on the physiology of the plant. The question of the essentiality of copper was a case in point. SOMMER, (**9**) and LIPMAN and MACKINNEY (**6**) have independently demonstrated the necessity of copper for the the vegetative growth and reproduction of higher plants. It was recently suggested, however, that the observed response to copper may have been due to an indirect influence, *i.e.*, its antagonistic or balancing effect on other elements (**8**). Attention was also called to the desirability of confirming the specificity of copper and excluding the possibility that some other element capable of existing in different valence states, could replace copper (**2**).

The specific and direct effect of copper on the growth of tomatoes was demonstrated as follows: Typical copper deficiency symptoms in tomatoes (very much stunted growth of shoots and exceedingly poor root development, dark bluish-green color of foliage, curling of leaves, and absence of flower formation) were produced by growing the plants in culture solution in two-liter Pyrex beakers, using purified salts and redistilled water (**12**). (Copper deficiency symptoms could be produced only if water redistilled over a Pyrex condenser was used. Ordinary distilled water contained enough copper to satisfy the needs of tomatoes, even when purified salts were used.) The de-

velopment of the deficiency symptoms was prevented by supplying a plant with 2 gammas of copper as $CuSO_4$.

To test the possible antagonistic effect of copper on other elements, as distinguished from its direct effect on plant growth (9), plants were grown in solutions supplied in one case with only three microelements: boron, manganese, and zinc, and in another case with these plus 20 additional ones, including lead, mercury, arsenic, and selenium. It was found that the onset, type, and severity of copper deficiency symptoms were the same in both cases. The additional group of 20 elements included elements capable of existing in different valence states, such as molybdenum, vanadium, chromium, nickel, cobalt, titanium, and tungsten, none of which were found capable of replacing copper.

Leaves of young tomato plants showing copper deficiency symptoms were sprayed with a very dilute $CuSO_4$ solution (0.02 parts per million of copper). This brought about recovery and the resumption of normal growth of both shoots and roots. These findings, showing the direct effect of copper on the plant as distinguished from its possible effect on the root environment, were interpreted as providing a final link in the chain of evidence of the essentiality of copper for the growth of tomato plants.

The small quantitative requirement of copper and other elements for plant life need not detract from an appraisal of their importance in the physiology of higher plants. There is no choice between essential elements. In that sense the fraction of a milligram of copper is just as indispensable as several hundred milligrams of a "major" element like potassium, since in the absence of either of them a tomato plant will fail to complete its life cycle. It is for this reason that the term "minor elements" frequently used to designate the group of elements essential for plants in minute quantity, should not lead to the inference that these elements occupy a position of secondary importance in the nutrition of higher plants.

The fact that most plants are injured by relatively very low concentration of microelements (for example injury to tomatoes was obtained from 2.0 parts per million of copper in the culture solution) adds interest to the search for the explanation of their function in the plant.

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ALKALOIDS FROM *SANGUINARIA CANADENSIS* AND THEIR INFLUENCE ON GROWTH OF *PHYMATOTRICHUM OMNIVORUM*

GLENN A. GREATHOUSE

(WITH ONE FIGURE)

Introduction

Sanguinaria canadensis L. is reported by TAUBENHAUS and EZEKIEL (**6**) to be highly resistant to the Phymatotrichum root rot. It has long been of interest on account of its alkaloidal content. DANA (**1**) isolated the alkaloid, sanguinarine, from this species in 1827. Since that time chelerythrine, protopine, and β- and γ-homochelidonine have been isolated from the rhizome and roots (**5**). For these reasons this plant seems an exceptionally good object for investigation in connection with the theory of alkaloids as a possible factor in the resistance of some plants to *Phymatotrichum omnivorum* (Shear) Duggar (**3, 4**). In the work reported here certain alkaloids were isolated from crude extracts of roots and rhizomes of *S. canadensis* and incorporated in various concentrations into the substrates ordinarily used for the pure culture of *P. omnivorum.* The effect of these substances on the growth and development of the fungus are discussed.

Materials and methods

Powdered rhizome and root tissue of *Sanguinaria canadensis* was purchased from Eimer and Amend Co. Two kilograms of this ground tissue was thoroughly extracted with acidified (acetic acid) alcohol, following the method of FISCHER (**2**) for the separation of the alkaloids. The procedure of extraction, separation, etc., is shown diagramatically in figure 1. The suggestions of the original paper were followed in separating protopine and chelerythrine from sanguinarine.

Results

Three alkaloids were separated from fractions BC and BS (figure 1). FISCHER found β- and γ-homochelidonine in fraction BSC, but in this study nothing of a crystalline form was secured; however, this fraction did yield a small amount of syrup of alkaloidal nature.

Alkaloids with the following physical properties and chemical composition were separated and studied for their effects on the growth of *P. omnivorum.* Alkaloid no. 1 (fraction BC and BS) crystallized from alcohol in colorless groups of needles, melting point 212° C.[1]; dissolved in alcohol, ether, chloro-

[1] All melting points are corrected.

form, forming solutions that were fluorescent. The hydrochloride was deep red in color. Chemical composition: Found,[2] C, 68.48 per cent.; H, 4.30 per cent.; N, 3.94 per cent. Calculated for sanguinarine ($C_{20}H_{15}O_4N \cdot H_2O$), C, 68.34 per cent.; H, 4.31 per cent.; N, 3.99 per cent. The concentration found for this alkaloid was approximately 0.2 per cent.

Alkaloid no. 2 (fraction BS) crystallized from alcohol in colorless prismatic leaflets, melting point 206° C.; readily soluble in chloroform, sparingly soluble in alcohol and ether. The hydrochloride formed by the addition of

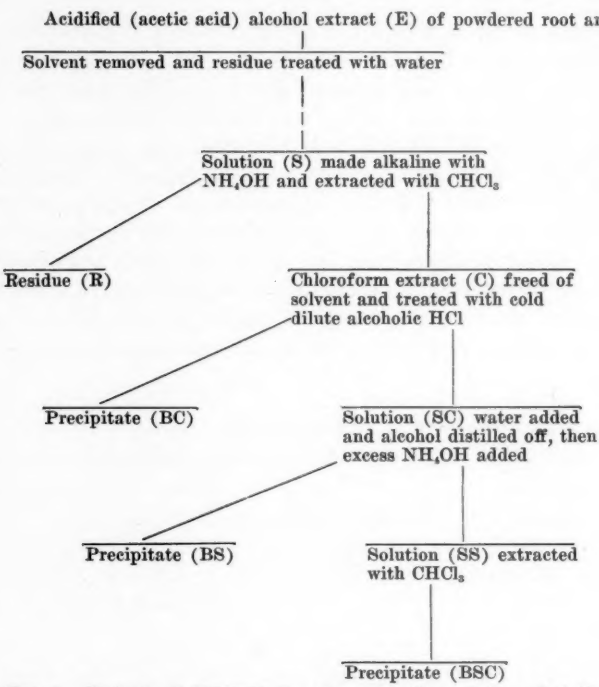
Acidified (acetic acid) alcohol extract (E) of powdered root and rhizome tissue
Solvent removed and residue treated with water
Solution (S) made alkaline with NH4OH and extracted with CHCl3
Residue (R)
Chloroform extract (C) freed of solvent and treated with cold dilute alcoholic HCl
Precipitate (BC)
Solution (SC) water added and alcohol distilled off, then excess NH4OH added
Precipitate (BS)
Solution (SS) extracted with CHCl3
Precipitate (BSC)

FIG. 1. Method of FISCHER for the separation of the alkaloids of *Sanguinaria canadensis*.

hydrochloric acid was citron-yellow in color, with needle-shaped crystals. Chemical composition: Found, C, 63.22 per cent; H, 5.05 per cent.; N, 3.63 per cent. Calculated for chelerythrine hydrochloride ($C_{21}H_{17}O_4N \cdot HCl \cdot H_2O$), C, 62.73 per cent.; H, 5.02 per cent.; N, 3.49 per cent. The concentration of this alkaloid was 0.1 to 0.2 per cent.

[2] Average of duplicate determinations made by Arlington Laboratories, Arlington, Virginia.

Alkaloid no. 3 (fraction BC) crystallized from chloroform by addition of a small amount of alcohol, formed monoclinic crystals, melting point 208° C.; very slightly soluble in alcohol, ether, benzene, and ammonium hydroxide; and soluble in acetone. Chemical composition: Found, C, 67.85 per cent.; H, 5.45 per cent.; N, 4.09 per cent. Calculated for protopine ($C_{20}H_{19}O_5N$), C, 67.96 per cent.; H, 5.42 per cent.; N, 3.97 per cent. The concentration of this alkaloid was 0.01 to 0.05 per cent.

The effect of these alkaloids on the growth of *P. omnivorum* was tested in the manner reported by GREATHOUSE and WATKINS (**4**). The results are presented in table I.

Discussion

Alkaloids having the physical properties and chemical composition of sanguinarine, chelerythrine, and protopine were isolated from *Sanguinaria canadensis*. All of these alkaloids, with the exception of protopine, were found to be present in the rhizome and root tissues in far greater concentration than that which prevents growth of *P. omnivorum*. The concentration of protopine found in the tissue was great enough to decrease fungous growth from 369 mg. to 81 mg. Sanguinarine completely prevented growth of the organism at a concentration of 2.5 p.p.m. Chelerythrine was not so toxic, permitting a growth of the fungus in three weeks of 3.4 mg. at a concentration of 10 p.p.m. Protopine under similar conditions yielded 81 mg. of

TABLE I

INFLUENCE OF ALKALOIDS ISOLATED FROM *Sanguinaria canadensis* ON GROWTH OF *Phymatotrichum omnivorum*

COMPOUND	DRY WEIGHT (MG.) OF FUNGUS IN NUTRIENT SOLUTION + FOLLOWING CONCENTRATIONS OF ALKALOID			
	100 P.P.M.	50 P.P.M.	10 P.P.M.	2.5 P.P.M.
	mg.	*mg.*	*mg.*	*mg.*
Sanguinarine	0	0	0	0
Chelerythrine	0	0	3.4	73
Protopine	81	137	308	356
Control = 369 mg.				

fungous growth at 100 p.p.m. This study clearly shows that alkaloids, even from the same plant, are not equally inhibitive to the growth of *P. omnivorum*.

Experimental evidence suggests strongly that the alkaloids in roots and rhizomes of *Sanguinaria canadensis* constitute an important factor in the resistance of this plant to Phymatotrichum root rot.

Summary

Sanguinarine, chelerythrine, and protopine have been isolated from roots and rhizomes of *Sanguinaria canadensis* by the method of FISCHER. Sangui-

narine has been shown experimentally to prevent the growth of *Phymatotrichum omnivorum* at a concentration as low as 2.5 p.p.m. Chelerythrine and protopine were less toxic than sanguinarine. There is considerable indication from this work that alkaloids play an important rôle in the resistance of *Sanguinaria canadensis* to Phymatotrichum root rot.

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SEASONAL SUGAR VARIATIONS IN ALFALFA

J. C. IRELAND

(WITH ONE FIGURE)

Ten years after planting, it is assumed that alfalfa plants have sufficient hardiness to be considered more than ordinarily vigorous, especially in the extremes of Oklahoma seasons. MEGEE (**2**) doubts that winter hardiness may be caused by physiochemical differences in alfalfas. He concludes that electrical conductivity indicates the degree of hardiness of alfalfa plants and that winter hardiness is an hereditary factor. On the other hand, GREATHOUSE and STUART (**1**), studying clovers, show that adaptability to cold is indicated by the unfreezable-freezable water ratio, osmotic pressure value, and the greater concentration of total sugars is in the hardier selections. Selecting seven row-plantings of ten-year-old alfalfa plants, the heritable phases of hardiness may be considered about equal. The object of this investigation has been to determine whether there is a seasonal variation in the sugar concentration and just how much environmental factors enter into the length of life of plants.

Weekly determinations of sugar have been made since October, 1937. A Zeiss hand sugar refractometer was used to make the readings. Sap was expressed from the crown of the plant by means of a heavy pair of pliers. A few drops of sap provide enough for a reading, and there is no deterioration or delay. The percentage reading on the scale of the refractometer is comparable to the total sugar concentration. Sampling was completed each Monday morning as a field routine. Soil temperatures were recorded with a thermograph. The bulb was placed vertically, and the record indicates the average for the top twelve inches of soil. Weekly averages were determined by means of a planimeter.

One hundred and forty alfalfa plants were transplanted to each row in the spring of 1928. The surviving plants have been used for this determination. Saskatoon came from the Experiment Station in Canada, and it was probably a selection among several by Dr. L. E. KIRK from his Asiatic collection. Forty-four of the original 140 had survived. Oklahoma Common was from a well-known seed grower in northern Oklahoma. Utah was from an original forty-year strain of that state, 57 of the original 140 surviving. Dakota, Cossack, and Grimm alfalfas came from North Dakota.

In order to determine the relative differences in young and old alfalfa plants, samples were taken from five reputedly hardy alfalfas. These are recorded in table II, and the determinations were made during the last two weeks in May. The names of these selections indicate the source of the seed, which was planted in February, 1938.

TABLE I

CONCENTRATIONS OF SOLUBLE SOLIDS IN ALFALFA CROWN SAP

DATE	SASKATOON 1122	OKLAHOMA COMMON	UTAH 2561	DAKOTA 552	COSSACK U.S.D.A.	COSSACK 2441	GRIMM U.S.D.A.	AVE. PERCENTAGE
	%	%	%	%	%	%	%	%
Oct. 4	9.0	15.0	8.0	9.5	8.5	16.0	14.5	11.5
Oct. 11	12.0	8.0	6.0	6.0	5.0	12.0	10.0	8.4
Oct. 18	11.0	10.0	13.0	9.3	6.0	15.0	11.0	11.0
Oct. 25	11.0	11.0	17.0	14.0	11.5	13.0	15.0	13.2
Nov. 1	18.0	16.5	17.0	10.0	12.0	14.5	14.0	14.5
Nov. 7	17.0	13.6	20.0	18.0	14.6	17.6	14.0	15.5
Nov. 15	17.2	10.0	16.8	13.0	12.5	15.0	20.0	15.0
Nov. 22	12.5	16.0	10.5	11.0	14.0	16.5	20.5	14.4
Nov. 29	20.5	13.0	13.5	18.0	15.4	19.0	18.5	16.8
Dec. 6	13.0	6.0	12.4	13.5	11.0	11.5	18.0	12.2
Dec. 16	23.0	22.0	16.5	19.5	14.0	18.0	14.5	18.2
Jan. 4	20.5	13.5	14.0	17.5	13.0	13.5	21.0	16.1
Jan. 10	18.5	15.0	16.0	11.0	10.5	16.5	9.5	13.8
Jan. 17	11.5	7.3	8.5	12.0	7.0	9.6	7.5	8.6
Jan. 24	19.0	14.2	12.0	18.5	13.2	11.8	18.2	15.3
Jan. 31	11.4	14.5	18.0	11.8	15.5	17.0	16.6	14.9
Feb. 7	21.4	14.5	22.4	13.5	10.8	18.0	11.1	15.2
Feb. 14	9.2	12.0	13.8	13.0	13.2	10.6	12.8	12.1
Feb. 21	12.0	14.5	11.6	9.4	9.0	10.6	8.7	10.8
Feb. 28	13.2	8.4	13.0	10.4	13.6	8.0	12.2	11.4
Mar. 7	8.4	10.2	11.4	7.5	7.0	11.2	13.4	9.9
Mar. 14	8.6	8.6	8.2	6.4	7.0	13.2	8.2	8.6
Mar. 21	7.7	6.0	9.5	8.6	8.4	9.0	5.5	7.8
Mar. 28	9.4	6.2	7.0	7.6	10.0	7.8	12.8	8.7
Apr. 4	13.0	11.4	10.4	13.2	10.6	7.4	10.6	10.9
Apr. 11	12.4	11.6	14.2	8.8	13.0	10.2	12.8	11.8
Apr. 18	12.8	10.4	10.2	13.0	10.4	12.4	11.8	11.5
Apr. 25	8.0	7.8	6.4	9.8	6.8	5.4	7.6	7.4
May 2	5.0	5.8	8.4	6.4	9.8	6.8	12.0	8.0
May 9	18.5	11.2	14.4	11.8	9.8	6.0	14.2	12.2
May 16	7.6	6.2	12.0	9.4	7.4	9.6	13.0	9.3
May 23	4.6	3.8	8.1	10.2	8.4	5.3	4.5	6.4
May 30	9.4	8.6	9.0	8.8	10.8	9.2	7.6	9.1
Average percentage	12.9 ± .55	11.0 ± .51	12.4 ± .49	11.5 ± .43	10.4 ± .41	12.0 ± .47	12.8 ± .48	11.8
Standard deviation	4.88 ± .39	4.36 ± .36	3.98 ± .33	3.65 ± .30	3.47 ± .29	4.02 ± .33	4.08 ± .34	
Surviving hills from 140 in 10 years	44	35	57	50	38	47	48	

Results

Table I summarizes the readings of sugar concentrations in percentages of the total soluble solids.

The uniformity of sap concentration throughout the winter seems to be the most important factor in the length of life. Oklahoma Common is an example of a selection having comparatively low resistance. During a warm week, the first of December, the reading was 6 per cent. This was followed by one of the coldest weeks of the winter, and the reading was 22 per cent. During the last half of March, temperatures were unusually high, and the reading was about 6 per cent. An April freeze and snow storm brought the soil temperatures almost to freezing, and the cell sap concentration almost doubled. A glance at other selections for these periods indicates a less widely

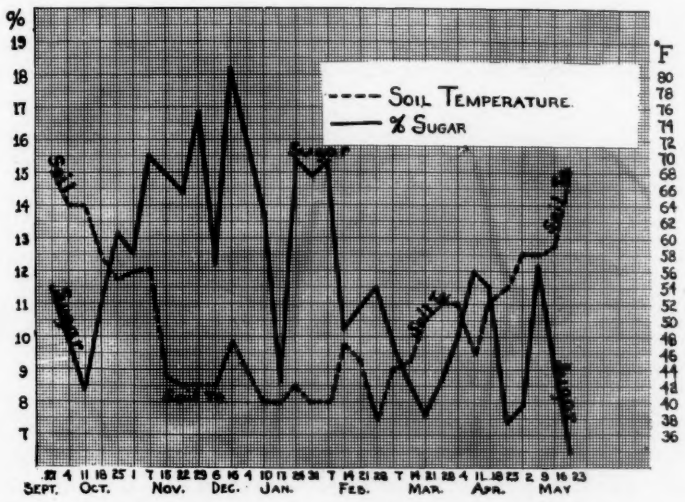

FIG. 1. Comparison of soil temperature with sugar concentrations of cell sap in alfalfa crowns.

divergent range. This is especially noticeable in Grimm, which seems to have a rather uniform concentration during the entire winter. Utah, with the greatest number of surviving ten-year plants, also has a comparatively small deviation. This observation is supported by the standard deviations from the mean of the several selections. GREATHOUSE and STUART (1) express their result with clovers: "... the adaptability of the red clover plant to winter conditions is closely associated with its metabolic rate." It is apparent that similar conclusions may be drawn in summarizing alfalfa.

During the last two weeks in May, similar determinations were made with a February, 1938, planting of five Minnesota varieties of alfalfa. Table II indicates that the sugar concentration does not differ materially from that of the older plantings.

TABLE II

YOUNG ALFALFA MAY, 1938

	MINNESOTA GRIMM	LADAK	IDAHO COSSACK	DAKOTA NO. 12	MINNESOTA VARIEGATED
	%	%	%	%	%
Sugar	10.2	7.6	7.6	8.7	9.2

A record of the soil temperature has been made for a number of years, and the averages for the winter of 1937–1938 are compared with the sugar concentrations of the alfalfa for the same period, in figure 1. It may be noted that November was an unusually cold month for Oklahoma. The sugar concentrations increased as the soil temperature dropped. A warm week during the first part of December was accompanied by a drop in sugar concentration. Rain in January also caused a decrease in sugar. During the spring months, the rise of temperature is followed by decreases in amounts of sugar.

Summary

1. The hardier selections of alfalfa have more uniformly higher concentrations of sugar than the less hardy.

2. The concentration of sugar varies inversely as the soil temperature.

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BRIEF PAPERS

RELATIONS OF PLANTS TO MINUTE DOSES OF INHIBITIVE SUBSTANCES

ERNEST S. REYNOLDS

It is generally recognized that minute doses of substances which are toxic in larger quantities may act in a stimulatory fashion, that is, they may cause the plant to accelerate one or more of its activities in excess of any stoichiometric relationship. Minute traces of mineral salts (**2, 4**) and auxins, bioses, vitamins, and other growth accessories (**1, 3, 7**) which are of biological origin, all seem to show this action and, above certain specific concentrations, the toxic action of representatives of most of these classes of materials has been demonstrated. The action of these substances, however, is much more complicated than the just mentioned facts would indicate. Living organisms not only react by stimulation or retardation of their individual activities, but also ultimately become accommodated to increased or decreased quantities of physical and chemical stimulative factors. This accommodation or acclimatization may become evident in various ways. THIMANN (**6**) has briefly called attention to a type of adjustment which is of very great significance in physiological studies. He says: "If roots are treated with auxin so that they are inhibited, and the auxin afterwards removed, their subsequent growth is accelerated. . . . The higher the concentration of auxin used, the longer it takes for this acceleration to appear, but on the whole the greater the acceleration is when it does come." Apparently referring to the same work WENT and THIMANN (**8**, p. 144) suggest that this reaction "presumably, is due to the rapid disappearance of auxin from the root, so that its concentration eventually reaches the accelerating level."

Some recent observations incidental to another investigation, upon rooting of oleander cuttings in solutions of hetero-auxin indicated the same type of reaction. Three sets of cuttings were made on April 18, some cuttings being placed in tap water (set C), some in 0.0013 per cent. hetero-auxin in water (set B), and some in 0.02 per cent. hetero-auxin in water (set A). Those in set B were given fresh solutions twice in the next three weeks, at the end of which time they were placed in tap water. They had developed callus tissue, but no root initials were externally visible. On April 20 the cuttings of set A were washed off and placed in tap water. The first cutting to show root development was from set C; and by May 9, 9 out of the 10 in this set had developed roots. On the other hand, by this date all of the cuttings of sets A and B had developed more callus and overgrowth of tissue than those in set C, but no roots. Those of set A had the greatest amount of overgrowth. By May 16 numerous roots were showing on all 3 cuttings in

set A, but in set B on only one cutting had a root appeared. In general the number of roots formed on the cuttings of check set C was less than on those of set A. By June 1, 6 of the 12 cuttings in set B had developed roots while the others had not rooted by June 14 and were discarded. Thus it is evident that the hetero-auxin stimulated tissue growth of the stem, and retarded root elongation. On the other hand more root initials were formed in the auxin-treated cuttings than in the non-treated, but at a considerably later date. The cuttings in the dilute solution which had been kept in this solution for three weeks were much retarded in root formation and suffered otherwise since half of them died during the experiment. Of those in this set which survived, several produced very large well-developed root systems, and some of the control cuttings likewise finally developed root systems which seemed the equal of some of the auxin-treated cuttings. Since these cuttings were used in another investigation, exact quantitative results can not be given.

The internal mechanism by which this type of adjustment takes place is of course a part of the irritable quality of protoplasm, but probably in each instance the detailed reactions are specific and different. In the reporting of experimental results and in making deductions from them, this type of reaction seems to have been given little if any consideration. It seems probable to the writer that this same type of adjustment would be likely to take place whenever there is a quantitative change in any of the stimulative factors of the environment, within the life-limits of the given factor.

The writer (**5**) formerly called attention to an accommodation of plant growth to a toxic substance in the case of a fungus, *Fusarium lini,* under the action of KCN. The initial growth of the fungus was retarded and the later growth accelerated, roughly in direct ratio to the increased concentration of the cyanide. More recently a preliminary inhibiting action followed by stimulation has been reported by WRIGHT and ANDERSON (**9**), in the action of water soluble derivatives of 1, 2, 5, 6-dibenzanthracene on the growth and glucose utilization of *Fusarium lini.*

WENT and THIMANN (**8,** p. 143) state that the inhibition of roots by auxins "is not comparable with that produced by toxic substances" but they do not elaborate upon this. The inhibition and later stimulation of *Fusarium lini* by KCN seems to be similar, at least in certain aspects, to this root reaction to auxin. Presumably more of the KCN remained present in the cultures in which the highest concentration had been used than in the lower concentrations, while in the auxin treatment of the cuttings the external auxin solution was completely removed before stimulation appeared. This might not have been necessary if a more dilute solution had been used since other experimental results show that the auxin is stimulative in much more dilute solutions than were used. It is possible that above the range of

immediately stimulative concentrations and below the completely inhibitive ones there would be a range of concentrations in which the roots would become adjusted and finally stimulated.

In any case, this preliminary inhibition and final stimulation of plant tissues by toxic substances, thus indicating an accommodation of the organism to them, points to the necessity of a more careful consideration of this phenomenon in physiological experimentation.

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CONDITION OF CHLOROPHYLL IN THE LEAF[1]

O. L. INMAN AND MARIE L. CROWELL

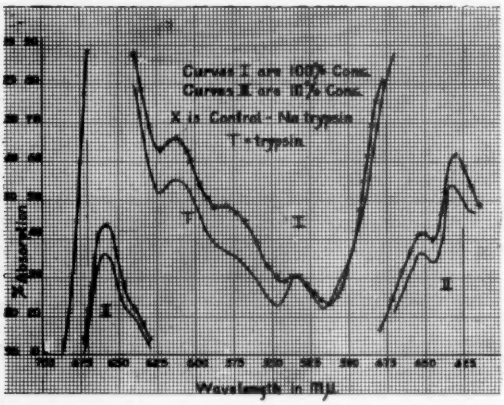
(WITH TWO FIGURES)

Extensive study of the microscopic structure of chloroplasts has been carried out by many workers. While this has given us considerable information about the probable nature of the condition of the chlorophyll pigments in the chloroplast there is still much to be explained.

The present experiments were undertaken for the purpose of investigating the physical and chemical state of the chlorophylls in the leaf.

Trifolium repens leaves were ground with sand, extracted with buffer solution, filtered, and centrifuged so that only the colloidal material was retained as the triturate. Using this triturate it is easy to recognize when the chlorophylls are losing the magnesium from the molecule: first, by the proportional increase of the intensity of the 534 mμ absorption band (ether as solvent) as compared with the other bands; and later, by the shift of the 662 mμ band toward the red end and appearance of a typical pheophytin spectrum.

Figure 1[2] shows the effect of adding 0.15 gm. of a crude trypsin (Eimer



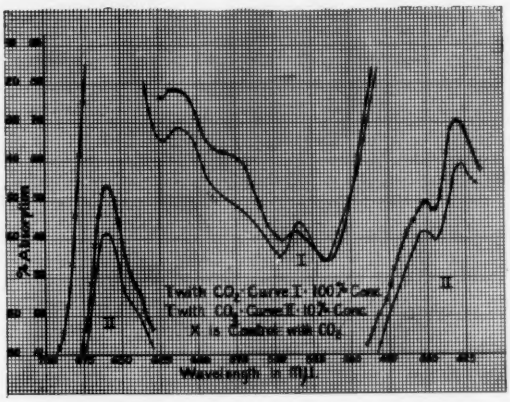
and Amend B 54) to 75 ml. of triturate and letting stand for 12 hours at 25° C. After the trypsin acted, the triturate was extracted with acetone and the chlorophylls transferred to ether by means of water. It is clear that the intensity of the 534 mμ band is proportionately greater with the use of trypsin.

[1] From the C. F. KETTERING Foundation for the Study of Chlorophyll and Photosynthesis.

[2] The curves for figures 1 and 2 were made by Dr. V. M. ALBERS (ALBERS, V. M. A recording photoelectric spectroradiometer. Jour. Opt. Soc. Am. 28: 121–123. 1938.).

The use of 0.0375 gm. and 0.075 gm. of trypsin clearly indicated that the degree of intensity of the 534 mμ absorption band was dependent upon the amount of trypsin added if the time, temperature, pH, and the amount of the triturate remained constant.

Figure 2 shows the same experiment except that carbon dioxide was



bubbled through the triturates one hour before the chlorophylls were extracted. It is evident that the 534 mμ absorption band in the triturate containing trypsin is proportionately more intense than the control after the passage of carbon dioxide into the triturates. The pH of the triturates at the beginning of the experiment was 5.6 (measured with a glass electrode) and remained about 5.6 in both the control and the one to which trypsin had been added. After bubbling carbon dioxide through the control and the trypsin-containing triturate the pH was 5.0. An attempt was made to bring about the same increase in intensity of the 534 mμ band in the control by bubbling carbon dioxide through the triturate three hours. This further addition of carbon dioxide from a tank did not produce detectable change in the intensity of the 534 mμ band or bring the pH lower than 5. These experiments were repeated with NORTHRUP'S crystalline trypsin and gave the same kind of results. Similar results were also obtained when triturates with pH 6.8 were used as starting material. In this case the pH after adding trypsin and without trypsin was 6.4, and yet the 534 mμ absorption band became more intense in the triturate where trypsin was used.

It seems fair to conclude from these experiments that trypsin causes the Mg to be freed from the chlorophyll molecules with the formation of pheophytin, and makes the chlorophyll molecules of the triturate more susceptible to the diplacement of the Mg by means of the addition of carbon dioxide. It is also clear that it is not just a matter of the change in the pH of the triturate

since that would naturally form pheophytin if kept too far on the acid side of neutrality for a period of time such as twelve hours at 25° C. It must also be clearly understood that no pheophytin formation has been observed when the triturate is definitely alkaline.

One might propose several explanations for these reactions. It appears that the action of trypsin on the substrate, to which the chlorophylls must be closely bound by adsorption or chemical combination, is acted on by trypsin, and the hydrogen-ion concentration of some allied substance is so altered that pheophytin begins to form; yet no lower pH is recorded. The most obvious substrate to be thought of is a protein. If this is true it may be a good assumption to consider the chlorophyll pigments as being bound to their substrate through a Mg-protein linkage. There is, however, another assumption which is being tested: will the trypsin not hasten the release of Mg from pure colloidal chlorophyll by acting directly on the magnesium-nitrogen linkages in the chlorophyll molecule? If this were true, then the assumption of a linkage between chlorophyll and a substrate native protein would not be so obvious.

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AN AUTOMATIC PLANT IRRIGATOR AND RECORDER

C. N. JOHNSTON AND O. A. ATKINS

(WITH ONE FIGURE)

Many forms of automatic irrigating equipment have been used by investigators growing plants in small containers. Most of these units have been devised to keep the gross weight of plant and container constant. To achieve this, water is added to the soil in small amounts as the plant consumes it. As a result the plant is often assumed to be growing under specified soil-moisture conditions but the facts contradict the presumption because it has been shown[1] that water added in this manner usually wets but a limited portion of the soil in the container to field capacity. The rest of the soil in the container will be much drier than the field capacity. The apparatus described below will automatically supply the soil mass within the pot with sufficient water to bring all the soil contained therein to the field capacity. In addition, a counter operated by the mechanism records the number of irrigations applied.

Figure 1 shows the apparatus with a cotton plant in a 10-quart container. It is seen that the apparatus is constructed from material usually found about the laboratory. The operation of the equipment is as follows: As pictured, the plant may be presumed to have been irrigated recently. The pot (no. 9) is heavy and has raised the counterbalance weight A against the stop, a nut on a machine screw soldered on the strap-iron leg running horizontally to the left of the top of the pot. As the plant removes water by transpiration the pot becomes lighter and at a predetermined weight, the counterbalance A will outweigh the pot. When this occurs, the pot will be lifted slowly till the catch B, pictured on the top of the shaft C in front of the pot, is released. When this occurs the water container E on the vertical wood support D at the left tilts forward swinging about 30 degrees and pivoting on the copper tube located close to the bottom of the can, and slightly back or to the left of the center of the container. When E has tilted, the syphon hose running across its front becomes primed and the water held in the container is discharged rapidly into the pot which becomes heavy and sinks till the stop again holds it. With the almost complete emptying of E, its weight is reduced so that the weight holder F, fastened to an extension of the pivoting tube, outbalances it and swings it back into vertical position. When E is vertical, the pivoted catch B on the other end of the linkage re-engages to hold E upright till the next release by the pot, which will not occur till the plant has used the soil moisture down to the desired limit. The small shot weight container attached to shaft C aids in balancing the linkage between

[1] VEIHMEYER, F. J. Some factors affecting irrigation requirements of deciduous orchards. Hilgardia 2: 125–291. 1927.

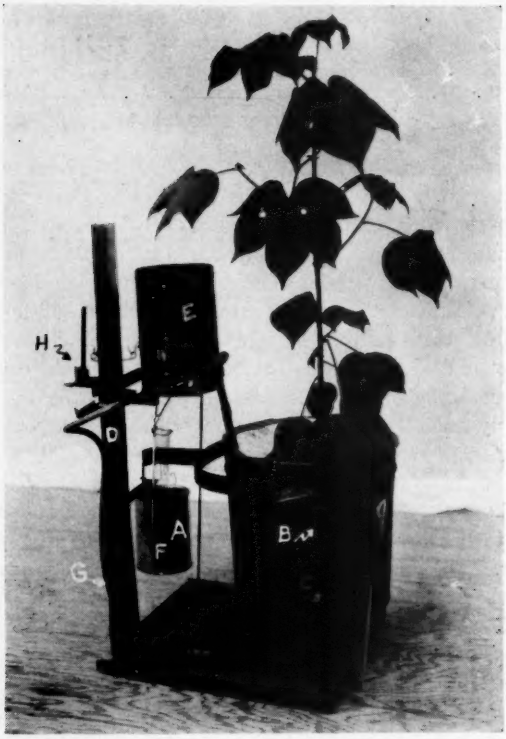

FIG. 1. Automatic irrigator and recorder with cotton plant.

the water container and the catch. The water supply to E comes from a constant level source such as a Mariotte flask. It enters the pivot pipe through the hose G running down the wooden shaft D and runs slowly through a 1/16-inch hole in the copper tube inside the can. The counter H is located on the stick supporting the water container and is operated by the motion of that can. As the can falls forward, the thick flat plate just below the copper shot tube to the left of the supporting stick is swung horizontally by the linkage and carries shot over to a hole in the plate below. This shot drops into the basket placed just underneath this plate. No shot escapes the shot tube during this motion, because the pivoting plate is wide enough to remain in sliding contact with the bottom of the tube. When the water container E is swung back to vertical, the moving plate on the counter is moved back so that the hole for the shot falls directly under the shot tube and one shot drops into position for the next cycle.

The correct volume of water to be added for each irrigation is regulated by moving the tilting water container E up or down on the supporting stick, keeping the lower edge of the syphon just above the required water level. Small adjustments in the height of the water container may be compensated at the bottom of the vertical link dropping from it. Changes larger than an inch require the use of different length vertical links. Otherwise all parts are standard. Hacksaw blades provided satisfactory pivoting surfaces for the operation of the balance system for the pot. They were soldered to the bracket supports. The ratio of loading of pot to counterbalance weight was 5 to 1, so the pivot arm lengths of pot and counterbalance can were in the ratio of 1½″ to 7½″. While operating a large number of these irrigators, a carburetor float valve may be substituted for the Mariotte flask, using the domestic water supply as a source, with satisfactory results.

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NOTES

Summer Meeting.—The summer meeting of the American Society of Plant Physiologists at Milwaukee, and at Madison, Wisconsin, will be held on June 20–22, 1939, in connection with the A.A.A.S. Registration headquarters will be in Milwaukee, and the first sessions will be held there. Other sessions have been arranged to be held at the University of Wisconsin. We hope that a large number of members and friends of the Society will be present on the opening day.

The headquarters will be at the Hotel Schroeder. In Madison, accommodations may be obtained at the Belmont Hotel and Cardinal Hotel at $2.00 and up, and at the Park Hotel and Loraine Hotel at $3.00 and up. Those who are driving cars to the meetings will find the large and well-appointed Monona Tourist Cabin Camp convenient. It is located on route 51, east of Madison, and may be approached from route 18 by a turn to the right, or from route 30 by a turn to the left, on reaching route 51 coming west from Milwaukee. Accommodations with breakfast will be available for a limited number in a lake-shore dormitory. If you desire a reservation, communicate with D. L. HALVERSON, Chadbourne Hall, Madison.

The program for Tuesday at Milwaukee presents a symposium on photoperiodism during the morning, and a general session for short papers in the afternoon. On Wednesday and Thursday at Madison invitation programs, inspection trips, and a picnic provide ample opportunity for scientific and social enjoyment. Come, and bring your friends along.

Western Section.—The fourth annual meeting of the Western Section of the American Society of Plant Physiologists will take place at Palo Alto, California, during the week of June 26 to July 1, 1939. The program will include three symposia: one in joint session with the Botanical Society on *Translocation of Solutes in Plants,* with T. G. MASON, Trinidad, British West Indies as chairman; one on *Growth* with F. W. WENT, California Institute of Technology as chairman; and one in joint session with the Western Society of Soil Science. Three half-day sessions are reserved for the reading of short papers.

In addition to the biologists' dinner on Wednesday night, there will be an informal dinner for plant physiologists on Friday evening. On Saturday morning there will be a trip to the laboratories and greenhouses of the Division of Plant Nutrition at Berkeley. Professor D. R. HOAGLAND has consented to conduct this trip, and it affords an excellent opportunity for visiting plant physiologists to see this outstanding laboratory.

Rooms and meals will be available on the Stanford campus. Those who

desire to avail themselves of the organized service for visitors may address Miss ANASTASIA DOYLE, Director of Residence for Women, Box 1772, Stanford University. Charges will be $2.00 per day per person up to 3 days, and $1.75 per day for 4 or more days.

Changes in Proofs.—The costs of making changes in the proofs of manuscripts are always large enough to be a considerable factor in the expenses of publication. The method of computing such costs is being changed to make possible the assessment of such costs against individual authors. Hitherto the charges have been made on an hourly basis, a method which did not facilitate computation of author responsibility. A new arrangement based upon the number of lines reset will make it easy to compute the changes in each paper. The charge is 12 cents per line for resetting galley and page proofs. We hope that authors will do at least an equivalent amount of thinking before they order changes made in their proofs. Actual errors must be corrected, of course; but the insertion of words, or omission of words, may cause many lines to be reset. If authors will retain the manuscripts until they are sure of just what they desire to say, if qualifying words and phrases are inserted or deleted in the manuscript before it is edited, most of the necessity for revision will be obviated.

Drawings.—Attention is again directed to the size of drawings sent with manuscripts. It is inadvisable to submit any drawings larger than the typewritten page. If made larger, they should be reduced photographically before they are sent in for consideration. A clear photograph not over 4.5 inches in width will give the author an idea as to whether the lines, letters, and numerals are large enough for successful reproduction. Many drawings arrive in dog-eared and torn condition because they are made on paper larger than the manuscript and not properly protected from damage. If the drawings are to be submitted to readers, photostat copies should be included for that purpose, so that the originals may be safely preserved against loss and damage while being circulated to the readers of manuscripts. Cooperation on the part of all authors is requested.

Errata.—Several errors occurring in volumes 12 and 13 of PLANT PHYSIOLOGY have been reported, which seem to the editor important enough to need correction.

VOLUME 12, plate opposite p. 565, the date of birth of HANS MOLISCH should read December 6, 1856.

p. 216, line 6 from bottom, for "July 12, 1856" read December 6, 1856.

VOLUME 13, p. 394, line 2, for "(1 p.p.m.)" read (5 p.p.m.), and for "(0.15 p.p.m.)" read (0.75 p.p.m.).

p. 397, to tabulated optimal synthetic solution add:

$MgSO_4$	35.00 mg.

Physical Methods Committee.—The Physical Methods committee of the American Society of Plant Physiologists has been reorganized to continue the important work which has been undertaken by this committee. Occasional reports will be prepared covering fields where the need for more refinement of techniques, or better understanding of principles and practical difficulties, is greatest. The committee was first organized in 1930, and has contributed a number of papers in the interests of better science. The following contributions are noted: *Light Sources and Light Measurements,* by HARDY L. SHIRLEY, 1931; *Osmotic Quantities in Plant Cells in Given Phases,* by A. URSPRUNG, 1935; *Methods of Research on the Physical Properties of Protoplasm,* by W. E. SEIFRIZ, 1937; *Conductivity Measurements of Plant Sap,* by GLENN A. GREATHOUSE, 1938.

Professor R. B. WITHROW, Purdue University, is chairman of the committee, and the other members are K. C. HAMNER, University of Chicago; B. S. MEYER, Ohio State University; O. L. INMAN, Antioch College; and S. T. DEXTER, Michigan State College. EARL S. JOHNSTON, of the Smithsonian Institution, former chairman of the committee, will serve in an advisory capacity.

Tree Growth.—In this volume MACDOUGAL brings together the results of many years' work with his dendrographs and dendrometers, as well as a store of related information on the growth of numerous Californian and other trees. One cannot but marvel at the energy with which the veteran, now in his 74th year, continues his researches. The most detailed study is devoted to the species *Pinus radiata,* the Monterey pine. Progressive changes in diameter of trunk and roots at different points along the axis, initiation and cessation of cambial activity, height increment of stem and apical growth of roots, the effects of partial defoliation at different seasons—all are here. The author tries to correlate growth with the factors which might control it, such as temperature, water supply, and hormone production. In this connection some experimental data on water contents and further microscopical observations of cambium would have been more convincing than mere inference, but even MACDOUGAL cannot tackle all sides of the problem.

Dendrograph records are of fundamental importance in many phenomena of tree physiology. The daily fluctuations of diameter which they reveal have long been regarded as the best evidence in favor of DIXON'S cohesion theory of sap ascent. With reference to the theory of hormone regulation of cambium action, the author's findings are "consonant with the conception of hormonic control of growth by auxins from the buds" as regards diametral growth of the trunk and perhaps of the proximal portion of the root; but they are opposed to such control of the distal portion of the latter in which cambial activity is more related to growth of root apices. Photosynthetic activity is

also illuminated by growth records considered in conjunction with leaf counts. The author has estimated the amount of wood formed per leaf of Monterey pine. One tree in its tenth year produced enough wood to form a layer 1.3 mm. thick over each leaf. The photosynthetic activity of a century old pine is estimated at 400 million leaf-years.

Of Californian superlatives the book has its share. There seems to be no doubt in the author's mind that the epithets oldest, largest, tallest, and fastest growing, all pertain to trees of the state. It is perhaps not surprising that *Pinus radiata,* his first love, to whose charms he has devoted so many years, is credited with forming cellulose at a higher rate than any other tree, but one wonders if BRADLEY'S *Albizzia moluccana,* with a growth from seed to an average height of 86 feet and a diameter of 9.75 inches in 7 years,[1] or some of the new hybrid poplars are also in the running.

There is so much of interest in this book that one is disproportionately irritated by the few flaws that mar it. Many of the tables, particularly those (such as xxviii) dealing with dendrometer readings, could have been rendered much more intelligible. Actual errors are few and far between; the figure at the bottom of p. 90 should be 0.000025 mm.; the height level in larch (p. 86) is surely not 27.5 M. (92 ft.).—G.W.S.

The volume is published by the Chronica Botanica Co., Leiden, 1938, and contains vi + 240 pp. The price is 7 guilders.

The World of Plant Life.—An excellent book about plants that will delight the layman as well as the connoisseur botanist is Dr. CLARENCE J. HYLANDER'S *The World of Plant Life* which comes from the Macmillan Co., New York. Profusely illustrated with good drawings and unusual photographs, the book leaves litfle to desire for beauty and attractiveness. The author, moreover, has a gift for popular writing which we hope he will exercise in other ventures, for botany has too few worthy advocates before the bar of public opinion. Books of this type can do untold good in public education.

The order of presentation follows fairly conventional lines of evolutionary sequence. There are five parts devoted to: the thallus spore plants; the leafy spore plants; the naked-seed plants; the fruit-seeded dicots; and the fruit-seeded monocots. The author uses the technical terms for these sections of the plant kingdom, but in parentheses. Each part is subdivided into chapters dealing with smaller groups within the main sections, and the author presents the entire classification in 10 phyla. There are 46 chapters, and all of them give one thrilling excursions into the fields of knowledge. Take the chapter on the cacti, for instance. Every cactus enthusiast will enjoy the 25 pages of description and exquisite photographs and be hungry for more at the end.

[1] Indian Forest. 48: 637–640. 1922.

One could wish nothing better than that young students of botany might be given such fare as this along with their stodgy and insipid courses in botany. It would add such spice and flavor as to make the study of plants a genuine pleasure while leading to the important fields of investigation. Every school library should possess it, and individuals will prize it in their private libraries. The check list of plants according to phylum at the end of the work (48 pages) may be used by classes to keep a record of their field experiences. A selected bibliography and an index complete this unusual work. The editor feels that this book is an outstanding contribution to popular learning, and while it is somewhat expensive, $7.50 per copy, it is worth a great deal more.

Annual Review of Physiology.—Our readers are familiar with the *Annual Review of Biochemistry* which has achieved an outstanding place among annual reviews. We take pleasure in introducing to our members the first number of *Annual Review of Physiology,* which has been edited by Dr. JAMES MURRAY LUCK and his colleague Dr. VICTOR E. HALL, both of Stanford University. The aim has been to attempt a critical appraisal of contemporary work in definite fields, to analyze and interpret the most significant contributions, rather than to include mention of all the papers appearing in physiological journals. Such reviewing, well done, assists in the integration of science, and is necessary to effective progress in physiological research. There are 24 reviews in this first volume: Permeability, by M. H. JACOBS; the biological effects of radiation, JANE HOWELL CLARK; physiological aspects of genetics, G. W. BEADLE; developmental physiology, JOSEPH NEEDHAM; growth, C. B. DAVENPORT, with OTTO RAHN, MARY E. MAVER, H. W. CHALKLEY, and D. M. PACE; temperature regulation, ALAN C. BURTON; energy metabolism, JOHN R. MURLIN; the peripheral circulation, H. C. BAZETT; respiration and its adjustments, ROBERT GESELL; muscle, EMIL BOZLER; the digestive system, A. C. IVY and JOHN S. GRAY; physiology of the liver, FRANK C. MANN and J. L. BOLLMAN; blood: physiology of formed elements and plasma; blood clotting, J. HAROLD AUSTIN; heart, J. A. E. EYSTER; electrical phenomena of the brain and spinal cord, HALLOWELL DAVIS; the spinal cord and reflex action, J. C. ECCLES; bioelectric studies of the excitation and response of nerve, DETLEY W. BRONK and FRANK BRINK, Jr.; the autonomic nervous system, JOSEPH C. HINSEY; the special senses, J. M. D. OLMSTED; physiological psychology: the conditioned reflex, ERNEST R. HILGARD, and miscellaneous topics, CALVIN P. STONE; kidney, HOMER W. SMITH; general and local anaesthesia, M. H. SEEVERS; applied physiology, D. B. DILL; and endocrine glands: gonads, pituitary, and adrenals, HERBERT M. EVANS.

Author and subject indexes supply means of rapid use of the material included. Plant physiologists who desire to maintain a well-balanced view

of the entire field of physiology will find it advantageous to browse the excellent material offered in these reviews. All of them bear the marks of conscientious and painstaking effort on the part of the reviewers. It is a fine companion series to the *Annual Review of Biochemistry*.

Copies may be purchased at $5.00 each, from Annual Reviews, Inc., Stanford University P.O., California.

Chemistry of the Amino Acids and Proteins.—An unusually fine treatise on the amino acids and proteins has been brought out through the efforts of Dr. CARL L. A. SCHMIDT, University of California. Sixteen contributors cooperated in the preparation of the book, which covers this vast field very ably. The first 10 chapters deal with the chemical statics of the amino acids and proteins, and the last 8 with chemical dynamics. Although the various chapters have been written by different authors, the work as a whole has been carefully planned with logical sequence of the chapters. A short account of the history of discovery in these fields is followed by chapters on the constitution and synthesis of the amino acids; isolation of the amino acids from proteins; preparation of amino acids and proteins; methods of analysis and reactions; relation of the amino acids to products of biochemical importance; peptides, peptidases, and diketopiperazines; chemical constitution of the proteins; molecular weights of proteins; certain chemical and physical characteristics of the proteins; and optical properties of amino acids and proteins. The dynamics includes chapters on the amphoteric properties, electrochemistry, combining properties, membrane equilibria, thermodynamics, relations to immunity, and rôle of proteins in nutrition. One cannot overstate the value of such a comprehensive treatment of the amino acids and proteins. The work contains over 1000 pages, and may be obtained at $7.50 per copy. Orders may be sent to the publisher, Charles C. Thomas, Springfield, Illinois. He deserves congratulations on having placed this valuable volume before the public.